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THE PHYSIOLOGY OF FISHES

Volume I

THE PHYSIOLOGY OF FISHES

Edited by
MARGARET E. BROWN
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Volume I

METABOLISM

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PREFACE

General knowledge of the physiology of fishes has lagged behind that of mammals and insects probably because of the greater difficulty of experimenting with aquatic animals. But fishes also are of economic as well as scientific interest and a great deal of work on them is widely scattered through periodicals published all over the world. This book consists of authoritative reviews of the present state of knowledge of various aspects of fish physiology; it is the most detailed book on this subject so far published in English (probably in any language). The authors have indicated gaps in knowledge and possible fruitful lines for further research.

Although intended primarily for those interested in fishes and fishery research, this book is also written for general zoologists and comparative physiologists. The physiology of fishes cannot be understood in isolation and in this work there are many comparisons with other vertebrates. These are of particular interest since fishes are aquatic and cold-blooded and represent a habit of life passed through during the evolution of the terrestrial, warm-blooded mammals.

Originally, "The Physiology of Fishes" was to consist of a single volume but in view of the mass of material available, the Publishers decided to issue two volumes, each of ten chapters. These are sub-titled "Metabolism" and "Behavior" but the terms are used in a very broad sense since most chapters are concerned with both. As Editor, I am responsible for the relative amounts of space allotted to different topics and for omissions.

I wish to thank all the authors for the cooperation and kindness they have shown throughout the project. I am deeply indebted to Dr. Ethelwynn Trewavas, of the British Museum (Natural History), for reading the proofs and checking the scientific names of fishes. I must also acknowledge gratefully the efficiency and kindness of the staff of the Academic Press.

MARGARET E. BROWN

Oxford
March 1957

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Chapter I: Part 1

THE AQUATIC RESPIRATION OF FISH

F. E. J. Fry

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I. INTRODUCTION

Respiration in water as opposed to respiration in air is conditioned greatly by two circumstances. These are the density of the medium and the greater dilution of oxygen in it. Water is approximately 800 times as

dense as air at the same temperature. Depending on whether it is fresh or salt, water at 20° C. for example will contain, at air saturation, 9.4 or 7.6 mg. of oxygen per liter as compared to approximately 250 mg. to be found at that temperature in a liter of air at sea level.

In association with the limitations on the access to oxygen imposed by the respiratory medium, the oxygen consumption of fish is less than that of the terrestrial vertebrates (Zeuthen, 1947). However the disparity is by no means as great as the mechanical difficulties of presenting a supply of oxygen to the respiratory surfaces would suggest. The gills of fish and their associated organs are extremely efficient in supplying oxygen from water of adequate oxygen content. When fish resort to aerial respiration, it ordinarily is to avoid water impossible as a respiratory medium and not to satisfy a metabolic level higher than that which other fish satisfy by aquatic respiration.

A. The Respiratory Gases

1. Oxygen

Values for the solubility of oxygen in water at various temperatures are presented in Table I. Air saturation values are the appropriate standard since the ultimate supply of oxygen for all respiration is drawn from the reservoirs of the atmosphere and equilibration of surface waters with the air sets, on the overwhelming majority of occasions, the upper

TABLE I

Oxygen in mg./l. in Fresh and Salt Water Approximately Saturated with Air at Sea Level [a]

Temperature °C.	Per Cent Chlorinity		
	0	10	20
0	14.7	13.0	11.4
10	11.5	10.3	9.0
15	10.3	9.3	8.3
20	9.4	8.5	7.6
30	7.8	7.2	6.4

[a] Adapted from Krogh (1941).

limit to the oxygen content of waters. Local enrichments above air saturation in small ponds and other quiet waters through the regeneration of oxygen by photosynthesis are of no significance to the general picture of oxygen supply. This is particularly so since such events follow the daily cycle of illumination and a substantial rise of oxygen during

the day is ordinarily followed by an equally marked drop during the night. Since the diffusion of oxygen through water is extremely slow, its vertical transport depends on the movement of the water itself. In consequence, wherever water is stratified, oxygen disappears from the depths to a greater or lesser degree due to its exhaustion by the organisms respiring there. Thus there is an oxygen gradient in water the reverse of that in air and one brought about by different means. Since the loss of oxygen depends on the balance between supply and utilization, waters also may become depleted of their oxygen even at the surface at sites of intense local enrichments of organic matter. Ground waters frequently are free of oxygen. The level of oxygen set by air saturation is governed of course by altitude and there are substantial reductions in the oxygen content of the waters of mountain lakes and streams compared with waters at sea level.

There are three general methods of determining the amount of oxygen dissolved in water. The one which has been used most is the Winkler iodometric method. In the laboratory this has been usually employed in its unmodified form and appears to be entirely satisfactory under most experimental conditions, particularly if its use is accompanied by tests for interfering substances (Ellis, Westfall, and Ellis, 1946). The iodine-difference modification of the Winkler method (Ohle, 1953) may offer a simple and exact means of eliminating the effects of interfering substances on the measurement of dissolved oxygen and should probably replace the unmodified Winkler technique in future work. Ohle's paper should be consulted by all workers interested in the determination of oxygen in water. Basu (personal communication) has demonstrated that the quantities of Winkler reagents ordinarily used are inadequate for the determination of oxygen levels much over air saturation. For work at these higher levels the quantity of the reagents should be trebled. Various adaptations of the Winkler technique to small samples have been developed, e.g. van Dam (1935), Fox and Wingfield (1938), Lindroth (1941).

The estimation of dissolved oxygen by the dropping mercury electrode has come into favor in recent years, e.g. Hayes, Wilmot, and Livingstone (1951), Spoor (1948). Oxygen may also be estimated gasometrically after extraction (e.g. Oesting, 1934).

2. Carbon Dioxide

Carbon dioxide does not occur in water in simple solution alone but enters into an incomplete reaction with water to become an acid, H_2CO_3. Carbonic acid then enters into a further equilibrium with the anions present to form carbonates and bicarbonates, the most important

of these being formed by calcium. For this reason the determination of carbon dioxide in water is a much more difficult problem than the determination of oxygen. Moreover it is necessary to distinguish between the total carbon dioxide, which includes that bound in all the stages of the equilibrium system, and the free carbon dioxide. The free carbon dioxide is the fraction which behaves like the carbon dioxide in the atmosphere with respect to its influence on respiration and is the

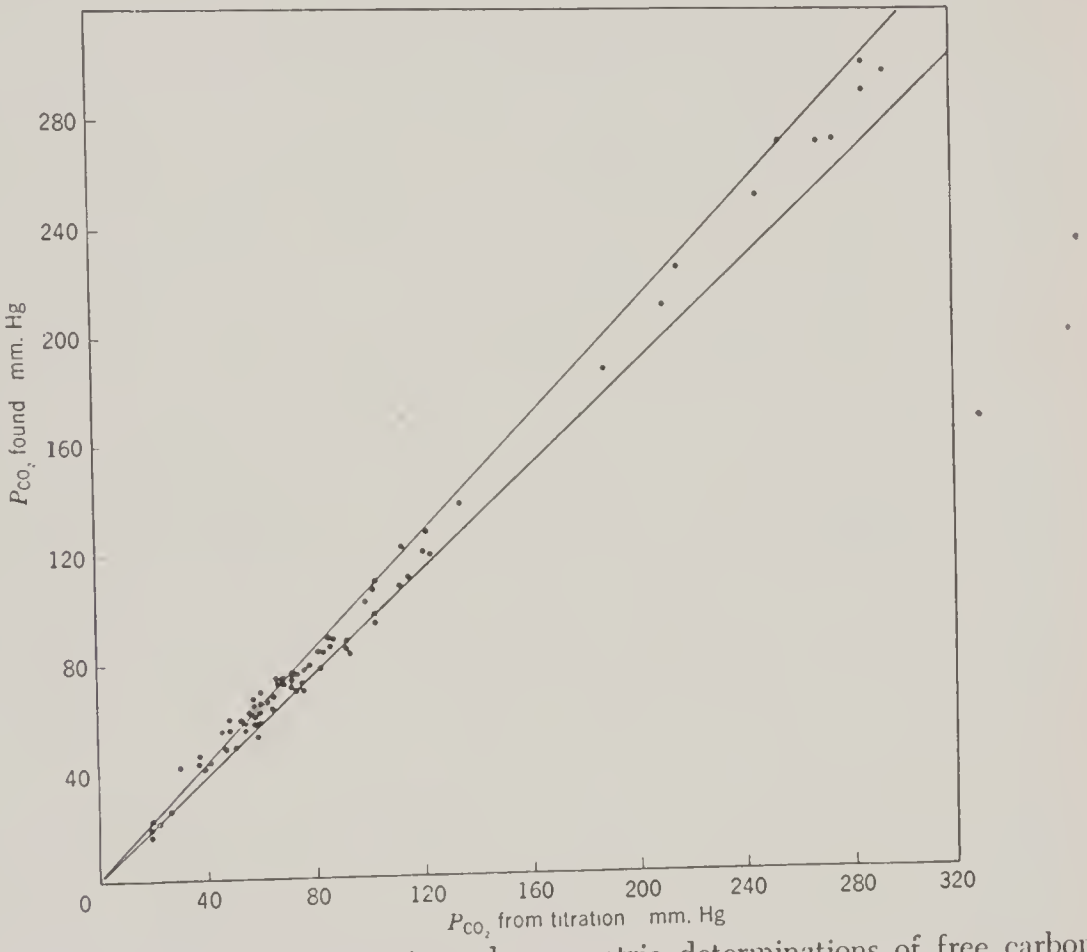


FIG. 1. Comparison of titrimetric and gasometric determinations of free carbon dioxide at 20° C. Data of V. S. and E. C. Black, part of those published in Black *et al.* (1954).

significant fraction to be determined if the effect of carbon dioxide on respiration is to be investigated. Free carbon dioxide can be determined with reasonable precision by the ordinary method of titration by sodium hydroxide using phenolphthalein as an indicator see "Standard Methods" (1946), or Sierp (1951), as is indicated in Fig. 1 where direct gasometric determinations on equilibrated air bubbles by Krogh's (1908) micro method are compared with titrimetric determinations. The determination of total carbon dioxide by titration is a much more difficult problem in measurements concerned with processes of respiration. The chief difficulty is that natural waters with any substantial degree of hardness

contain relatively large amounts of total carbon dioxide and the addition of respiratory carbon dioxide to this initial large content changes it very little. Thus the resulting estimate is ordinarily a small difference between two larger numbers. The large numbers, therefore, must be determined with the best possible precision. The ordinary titration with acid using methyl orange leaves much to be desired from this point of view. The best method for determining total carbon dioxide is therefore direct extraction in the Van Slyke apparatus (Hall, 1923; Henze, 1933; Oesting, 1934; Bosworth, O'Brien, and Amberson, 1936).

The lower limit of free carbon dioxide in fresh water is ordinarily fixed by equilibrium at the air–water interface although with photosynthesis it may drop below this value to zero. In the sea at the normal pH of sea water the partial pressure of carbon dioxide is about ¼ mm. Hg (Prosser *et al.*, 1950). The upper limit of free carbon dioxide in natural waters depends on the buffering capacity of the water concerned and on the intensity of anaerobic respiration.

3. Expression of the Content of Dissolved Gases

In air, pressure and density, except for a minor correction, are equivalent measures of the oxygen content over the normal environmental range of temperatures. Since the special effects of pressure, particularly with respect to diffusion relations and to the state of equilibrium reached in incomplete reactions, are so important in the oxygen economy of the organism, physiologists ordinarily express the oxygen content in terms of partial pressure. In water however to express all measures of oxygen content in terms of partial pressure is misleading. There is a much greater temperature effect so that for the same partial pressure the mass of oxygen dissolved in water decreases by 33% with a change in temperature from 10° C. to 30° C. In air the same change in temperature changes the mass by only 7% for a given constant pressure. However the greatest difference in the behavior of oxygen in the two media is in the ratio of pressure to density. In air at 20° C. a partial pressure of oxygen of 160 mm. Hg is associated with a density of 0.29 g./l. or a P/D ratio of 5.5×10^2. In fresh water, because of the relatively low solubility of oxygen, the corresponding ratio is 175×10^2, over 30 times as great. In sea water the ratio will be higher still. Because of this vast difference in pressure–density relations the density of the oxygen supply is more frequently the limiting aspect of this substance in water than is the pressure aspect. For this reason in all discussion here where questions of the oxygen supply are concerned, the oxygen content of water will be expressed as milligrams per liter. Only in cases where the pressure characteristics of the medium have special significance, such as in the

presentation of blood curves, will oxygen be expressed in terms of partial pressure.

For carbon dioxide the situation is simpler. The capacity of water for dissolving carbon dioxide is so very much greater than it is for oxygen that only the pressure aspect need be considered. In consequence the content of free carbon dioxide will always be expressed in terms of partial pressure here.

II. THE RESPIRATORY SYSTEM

The description of the respiratory system will be necessarily greatly curtailed here. The reader should consult the following papers (Baglioni, 1910; Winterstein, 1912–13; Woskoboinikoff, 1932; Leiner, 1938; Henschel, 1939; Krogh, 1941; Bitjel, 1949), if more than a casual knowledge of the subject is desired.

The respiratory apparatus in the myxinoids differs in many details from that found elsewhere in fishes (Rauther, 1937); no reference to these peculiarities will be made here. The gills of all larval cyclostomes are of course different from those of other fishes and will likewise not be dealt with.

The gills of fishes are associated with the anterior end of the gut being on the sides of lateral openings in the pharynx which lead from the digestive tract to the exterior. They are ordinarily enclosed in a branchial chamber the only exceptions to this being in the case of certain temporary larval gills which are external (Leiner, 1938). The water is ordinarily taken in anterior to the gills through the mouth or, in the case of those groups which possess them, through the spiracles as well and passes laterad through the gills and out through the external branchial openings.

A. The Branchial Pump

1. General Description

The structures which maintain the flow of water over the respiratory surfaces are the walls and floor of the oral cavity, the covers of the external respiratory apertures, and the branchiostegial apparatus. In the cyclostomes (Roberts, 1950) the resilient branchial basket together with an inertia effect produced by the forceful expiratory stream is responsible for inspiration. In this respect this group differs from the other vertebrates. In adult cyclostomes the flow of water is often tidal, and perhaps regularly so, being both taken in and ejected through the external branchial openings.

The intake and expulsion of water is usually an intermittent process

but in some instances, as in *Rhina* (= *Squatina*) (Darbishire, 1907), where the water is propelled by peristaltic waves of the gill cover, the inflow may be continuous. As in mammals the rate of the respiratory movements bears a general relation to size, small fishes making many movements per minute, large fishes few. At more or less regular intervals (Mines, 1913) the normal respiratory rhythm is interrupted, e.g. François-Franck (1906). In this interruption the direction of the respiratory current may be reversed, water being shot rather violently out from the

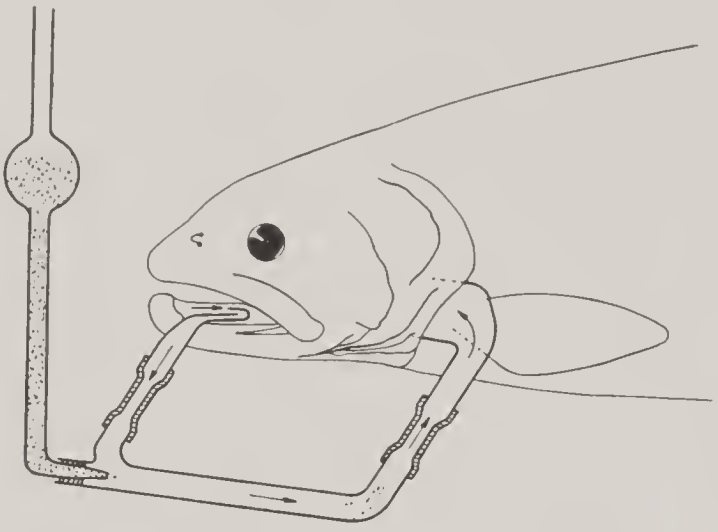


FIG. 2. Van Dam's demonstration of the continuous flow of water over the gills of fish (from van Dam, 1938).

mouth or spiracle (Rand, 1907) but it may be ejected with about equal violence through the external branchial opening (Bitjel, 1949). This behavior has been termed a gasping, coughing, or cleaning reflex and serves to rid the gills of foreign matter.

However, while the intake and expulsion of water are discontinuous processes, the flow of water over the gills is apparently usually continuous (Fig. 2). In teleosts, Woskoboinikoff and Balabai (1936, 1937) and van Dam (1938) have demonstrated that there is usually a higher hydrostatic pressure on the oral side of the gill than there is on the opercular side at all phases of the respiratory cycle. Balabai (1939a, b) extended these observations to the sturgeons and elasmobranchs. The branchial pump in all fishes but the Marsipobranchii therefore appears to be a combination of a pressure pump before the gills and a suction pump behind them. These relations are well described by Henschel (1939).

During this continuous flow across the gills, intake of water into the mouth and its expulsion from the external branchial opening take place over relatively short segments of the total ventilatory cycle. For example

in *Scorpaena porcus* (Hazelhoff, 1938) abduction of the operculum occupied 24 seconds while adduction took only 1 second. This unequal division of the phases of adduction and abduction is possible because the mouth is provided with valves and the gill covers with flexible extensions. The oral valves (see Gudger, 1935, 1946) check the backflow of water before the mouth is closed during the pressure pump phase when the oral cavity is being reduced in volume. The flexible extensions of the gill covers glide forward over the body wall as the chamber external to the gills is increased in size by the abduction of the gill covers and of the branchiostegial apparatus during the suction pump phase. The opening of the mouth and adduction of the external gill opening are only out of phase by about one-quarter of the cycle (François-Franck, 1906) thanks to the operation of these valves.

Fish are able to maintain a respiratory current, presumably by various compensatory maneuvers, in spite of considerable interference with the normal functioning of the apparatus (Lombroso, 1907; Henschel, 1939). Salmonoids of various species live successfully for years in spite of deformities which prevent the normal closure of the external branchial aperture (Osburn, 1910; Tchernavin, 1938).

The development of the branchial pump varies enormously from species to species. Baglioni (1908) partially classified the teleosts on the basis of their power to irrigate their gills as follows:

(1) Respiratory movements predominantly opercular—pelagic species.
(2) Branchiostegial apparatus more developed—species which rest on the bottom but are otherwise free swimming.
(3) Respiratory movements predominantly branchiostegial—bottom species.
(4) A miscellaneous group in which there is no true branchiostegial apparatus.

He also dealt with the elasmobranchs in a somewhat similar fashion.

To increase the volume of water moved, both the rate and the amplitude of the respiratory movements are increased. In *Salmo gairdnerii* van Dam (1938) found that the ventilation volume was increased predominantly (approximately four-fold) by change of amplitude. Increase in rate accounted only for an increase in respiratory volume of 1.3 times. In contrast he found that *Anguilla anguilla* increased the ventilation volume largely by increasing the respiratory rate.

2. Control of the Breathing Rate

As in other vertebrates, the respiratory rhythm is under control of the respiratory center and is modified by local reflexes which keep the mechanism functioning smoothly (Black, 1951). In fishes the effects of these reflexes are much more marked than they are in air-breathing

vertebrates. Powers and Clark (1942) found in three species of teleosts that sectioning of the afferent nerves from receptors in the gill regions immediately brought about cessation of respiratory movements. They consider this to be evidence of a dominant role of the reflex system. There was for years a controversy concerning whether or not the breathing rhythm of fishes was controlled solely by reflex reactions to currents of water. Reference to these papers may be found in Deganello (1908), Lombroso (1908), and in the earlier reviews on respiration (Baglioni, 1910; Winterstein, 1912–13). Finally van Rynbeck (1911) dismissed the matter as a "pseudo-problem."

Black (1951) has recently summarized the literature concerning the control of the respiratory rhythm, and presents a table showing the effect of change in the concentrations of the respiratory gases on the breathing rate in teleosts and elasmobranchs. The weight of the evidence indicates that teleosts respond to lowered oxygen and increased carbon dioxide by increased ventilation although the response is variable. With regard to elasmobranchs it is uncertain as to whether or not there is a response in ventilation at all but there is a response by increase in general activity. However, most experiments so far performed suffer because it is not certain that precautions were taken to ensure that the subject was in a state of quiet respiration before the experiment began. Since Black's summary, Wikgren (1953) reported an increase in the ventilation of *Petromyzon* (= *Lampetra*) *fluviatilis* in response to a decrease in ambient oxygen concentration. To Black's references should also be added von Holst (1934) who obtained evidence for control of the respiratory rhythm by a center in the upper region of the spinal cord or the lower region of the brain by suppressing external stimuli through the use of urethane. Some of the best evidence of central control of the respiratory rhythm is offered by the behavior of air-breathing fish which suspend movement of the opercula when in water containing certain concentrations of the respiratory gases, see Willmer (1934).

In quiet respiration certain gills may be allowed to stagnate. In elasmobranchs Darbishire (1907) reports that *Raja batis* inhales solely through the spiracles when at rest; these serve the first four gills chiefly. In activity, inspiration is carried on through the mouth as well. In the eel, van Dam (1938) sometimes observed ventilation of the gills on one side only during quiet breathing. Wikgren (1953) observed a gradation in the intensity of ventilation in the cyclostome, *Lampetra fluviatilis*, passing from contraction of only the respiratory section of the pharynx with a resultant transfusion of the gill pouches, through a contraction of the two or three anteriormost pouches, to full contraction of all pouches.

When fish are active, some irrigation of the gills is obtained as a

by-product of the forward movement. In certain pelagic species forward movement is essential to adequate respiration. Hall (1930) showed this to be true for *Scomber scombrus* which when closely confined could only load its hemoglobin to the extent of 11%. This species therefore has practically lost its ability to pass water over the gills by the ordinary respiratory movements.

Because of the density and viscosity of the respiratory medium together with the low concentration of oxygen in it, the cost of respiration is high. Van Dam (1938) reports a 70% increase of the resting metabolism when the ventilation volume increased four-fold in a rainbow trout exposed to water low in oxygen.

B. The Respiratory Surface

1. Structure of the Gill

The major seat of the interchange of the respiratory gases between the blood and the water is the gill, although the general body surface also often affords easy passage to oxygen and carbon dioxide. The gills in addition to their respiratory function are also secretory and excretory organs as will be discussed in later chapters. The pseudobranch in particular probably has no essential respiratory function.

It has long been agreed that the respiratory gases are not carried across the surfaces separating the water from the blood by any means other than diffusion. Indeed under special circumstances diffusion of oxygen may be from blood to water instead of from water to blood. Thus Cunningham and Reid (1932) hold that the high vascularity developed by the fins of *Lepidosiren* is to allow emission of oxygen to the eggs in the burrow. Krogh (1941) doubted this, speculating that in the rainy season there probably would not be any lack of oxygen in the water. However, he had apparently overlooked the report of Carter and Beadle (1930) that the oxygen content in the water in which the eggs developed was low. In *Synbranchus,* the general body surface of the incubating male is believed by Cunningham (1932) to perform the same function. The skin may also be important for the emission of carbon dioxide by air breathing species.

The general surface of the body, at least when not heavily armored is permeable to the respiratory gases but normally this is of no appreciable significance in aquatic respiration. Cutaneous respiration appears necessary to *Periophthalmus,* however, when it is confined below the surface (Haddon, 1889).

The gills (see Fig. 3) consist of a series of folds, the gill filaments, radiating dorso-laterad to ventro-laterad from the edges of the pharyn-

geal slits. The gill filaments are, with minor exceptions, marshaled into two vertical demibranchs anterior and posterior to the gill slit and each demibranch is supported, respectively, on the septum anterior or posterior to the gill slit. In the teleosts and other higher groups there is a reduction of the septa between the gill chambers and the gill filaments are free for two-thirds or more of their length.

The gill filaments bear on either side a series of transverse plates, the secondary lamellae. These are the significant sites of respiratory exchange (Plehn, 1901). The respiratory lamellae are thin walled and consist of a central open web of a single layer of supporting cells, the pilaster cells, the outer ends of which extend in processes (Keys and Willmer, 1932) which provide a basal connective tissue for the support

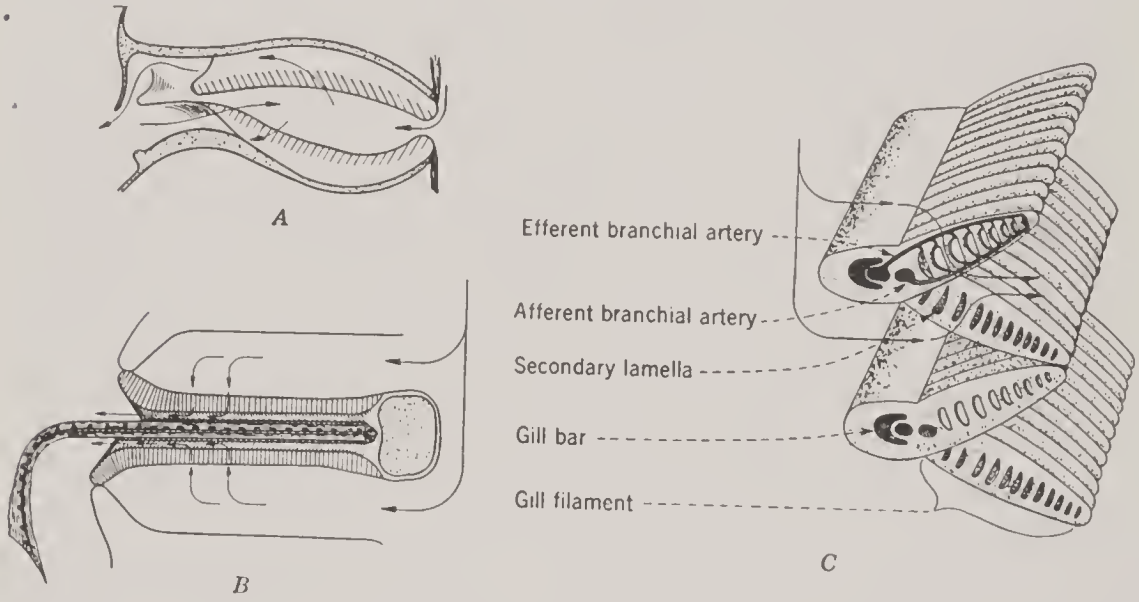


FIG. 3. Formalized segments of gills from the coronal aspect showing direction of the respiratory current. A, cyclostome; B, elasmobranch from Woskoboinikoff (1932); C, teleost from Bijtel (1949).

of the covering respiratory epithelium which consists of another single layer of cells. The blood lacunae thus formed in the lamellae link up the branches of the afferent and efferent branchial arteries associated with each gill filament. The role of the lamellae as carriers of the blood is passive. Krüger (1928) and Bijtel (1949) both reported there is no pulsation of the lamellae such as Reiss (1881) and Henschel (1939) believed took place.

The lamellae on adjacent sides of two neighboring filaments interdigitate and thus present a series of tiny channels through which the water flows in a direction perpendicular to the free edge of the filament. Direct observation in teleosts (Bijtel, 1949) has shown that the gill filaments of the aboral and oral demibranchs associated with a particular

gill slit are normally arched toward each other. Thus the water passes directly from the pharyngeal chamber to the epibranchial space through the myriads of tiny orifices presented by the intermeshing lamellae. During the coughing reflex the filaments are momentarily pulled wide apart by their intrinsic muscles (Bitjel, 1949). Wherever the gill filaments are not free from the septum, the lamellae do not extend entirely across the filament from the free to the fixed edge. Thus a channel is left which extends along the septum to the space between the gills and the external branchial opening. The water passes by this channel to the epibranchial space after passing between the interdigitated lamellae. The lamellae of the lamprey gill have a disposition similar to that found in the elasmobranch and a special arrangement of valves at the external branchial opening (Balabai, 1935) provides a unidirectional flow of water over them in tidal respiration (Fig. 3).

As a consequence of the structure of the gill the respiratory water is brought very completely into contact with the surfaces through which the respiratory interchange takes place and the system is very efficient with respect to the percentage of oxygen removed from the inspired water. The best available measurements (van Dam, 1938; Hazelhoff, 1938) place the utilization at from 50 to 80% under ordinary conditions. It would seem possible that the somewhat restricted passages for egress of the respired water in the elasmobranch made necessary by the retention of the septum would make their gills somewhat less efficient than those of the forms in which the septum has been eliminated. It does happen to be the case that the utilization of oxygen from the water respired was less in the single species of elasmobranch studied by Hazelhoff (1938) than in any of the seven species of teleosts he studied. Van Dam found the percentage of oxygen fell appreciably at higher ventilation volumes (down to 50% in *Salmo*).

Another anatomical feature that contributes greatly to the efficiency of the piscine gill is the arrangement of the blood supply. The disposition of the efferent and afferent blood vessels is such that the blood flows through the branchial lamellae in the opposite direction to that of the flow of water past them (van Dam, 1938). This counter-current system promotes the greatest exchange of the respiratory gases. The blood, just as it leaves the gills, comes in contact with the water bearing the highest content of oxygen and lowest content of carbon dioxide.

2. Quantitative Variation in Respiratory Area

The relative extent of the respiratory surface varies greatly from species to species. The most extensive series of observations is due to Gray (1954) whose data are included in Table II. Gray found a nine-fold

variation in the relative area of the gills in a series of marine fishes taken along the Atlantic coast of the United States. He also found a three-fold variation in the number of lamellae per millimeter of gill filament. Gray points out these differences are correlated with the degree of activity of the species as inferred from their general behavior and from the rate of

TABLE II
ESTIMATES OF THE RESPIRATORY AREA OF THE GILLS [a]

Habitat	Gill lamellae per Millimeter of Filament	Gill Area per Gram of Body Weight (sq. mm.)
MARINE TELEOSTS		
Scomber scombrus	31	1158
Poronotus triacanthus	31	598
Brevoortia tyrannus	29	1773
Mugil cephalus	27	954
Cynoscion regalis	27	373
Coryphaena hippurus	26	710
Stenotomus chrysops	26	506
Palinurichthys perciformis	24	506
Centropristis striatus	21	458
Prionotus carolinus	20	483
Roccus lineatus	19	302
Archosargus probatocephalus	19	328
Anguilla rostrata	19	302
Paralichthys dentatus	19	242
Tautoga onitus	18	392
Spheroides maculatus	16	470
Opsanus tau	11	197
INDIAN FISHES		
Water-breathing	..	152
Air-breathing	..	ca. 77
TEMPERATE FRESHWATER		
Micropterus dolomieu		
0.3 g.	28	740
837 g.	19	225

[a] Data from the following sources: temperate marine teleosts (Gray, 1954); Indian fishes, (Dubale, 1951); *Micropterus* (Price, 1931).

oxygen consumption of excised brain tissue (Vernberg and Gray, 1953). Dubale (1951) reported measurements of the gill surface of individual fish of 34 tropical species. These did not show the same wide diversity of area found by Gray and all had a lesser area than the toadfish, the species in Gray's series that had the least surface. Pütter (1911) lists gill areas of 58 to 295 mm.^{2} per gram for individuals of 7 species of fish.

Wikgren (1953) estimated that in a 100-g. specimen of the lamprey *L. fluviatilis* the relative area of the gills was 600 mm.^{2} per gram. Other isolated values are to be found in the literature all tending to the lower end of the range reported by Gray.

Dubale also measured the gill area of seven representatives of air-breathing species of fish. The gills in these were reduced to about one-half the area of those fish he investigated that had only aquatic respiration. Schöttle (1931) reported a similar reduction in the branchial surface in air-breathing gobies.

Price (1931) is the only worker who has carried out an extensive study of the relation of magnitude of the gill surface to the size of the individual. The gill grows both by the increase of numbers of filaments and numbers of lamellae on the filaments as these latter grow in length. The lamellae also increase in size as growth proceeds. Price reports interlamellar growth of the filaments resulting in a reduction of the number of lamellae per unit length of filament as the fish becomes larger. Other workers have not considered this to be the case, perhaps because they have not examined as extensive a size range of fish of one species as did Price who found that the greatest change in the number of lamellae per unit length of filament came early in life. Price found that the area of the gill surface in *Micropterus dolomieu* was related to the body weight according to the following formula, $G = 8.65W^{0.785}$, where $G =$ gill area in cm.^{2} and W the weight in grams. Thus the relation is intermediate between proportionality to surface area (Pütter, 1909) and to body weight (Reiss, 1881).

3. The Respiratory Surface for Embryonic Respiration

Embryos carry on their early respiration over the general body surface. Later in most cases a vascular network enveloping the yolk greatly extends the respiratory surface. Still later the median fin folds and the pectoral fins provide a further extension. Young *Synbranchus* (Cunningham, 1932) have large and vascular pectoral fins which drop off about 10 days after hatching. Many embryos including those of all elasmobranchs, dipnoans, *Polypterus*, and *Amia* develop threadlike external gills. Leiner (1938) classifies the conditions under which these appear as follows:

(1) When the pumping mechanism is late in appearing (*Acipenser*, *Cobitis*).
(2) When there is no vascular network over the yolk sac.
(3) When gills play no more than a minor role in the respiration of the adult.

The spontaneous movements of fish embryos, which begin almost

with the beginning of development (see Kuhl, 1940) break down diffusion gradients in the fluid within the egg capsule.

C. The Medium for Respiratory Transport

The blood of fish functions as a bearer of the respiratory gases essentially as does the blood of terrestrial vertebrates and the only aspect that will be dealt with here will be the range of variation in its characteristics for respiratory transport.

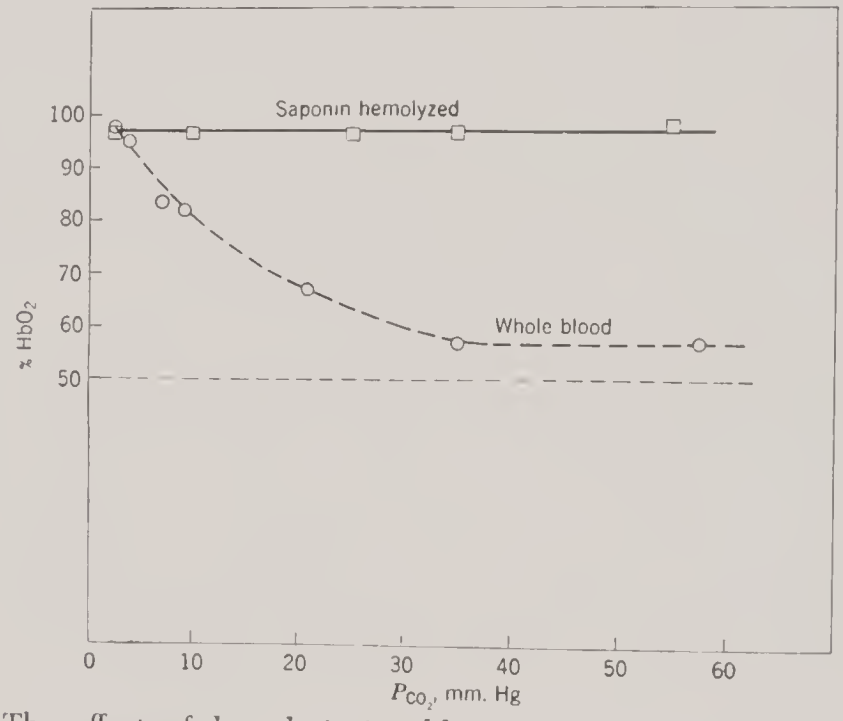


FIG. 4. The effect of hemolysis in obliterating the Bohr effect in fish blood (*Catostomus commersonii*) (from Black and Irving, 1938).

1. OXYGEN TRANSPORT

Variations in the oxygen capacity of fish blood are listed in Chap. II. Two other features of significance in oxygen transport, the ability of the blood to take up its load of oxygen at a given partial pressure of oxygen and the effect of carbon dioxide on the saturation of blood with oxygen show wide variation in fishes.

Black (1951) has emphasized the fragility of fish blood and that the integrity of the corpuscle is obligatory when blood gas relations are being investigated if results of physiological rather than biochemical import are desired. Black and Irving (1938) reported that hemolysis of the blood of *Cyprinus carpio* may be brought about by the use of potassium oxalate as the anticoagulant. Hamdi and Ferguson (1940) found similarly that fluorides, used to prevent glycolysis in mammalian

blood, had a hemolytic effect on fish blood. Figure 4 shows how hemolysis may entirely remove a marked Bohr effect displayed by whole blood. In consequence any substance used to prevent coagulation or suppress respiration should be chosen with care. Irving, Black, and Safford (1941), Black and Black (1946), and Ferguson and Black (1941) noted changes in the blood gas capacities of fish blood on storage; this indicates that

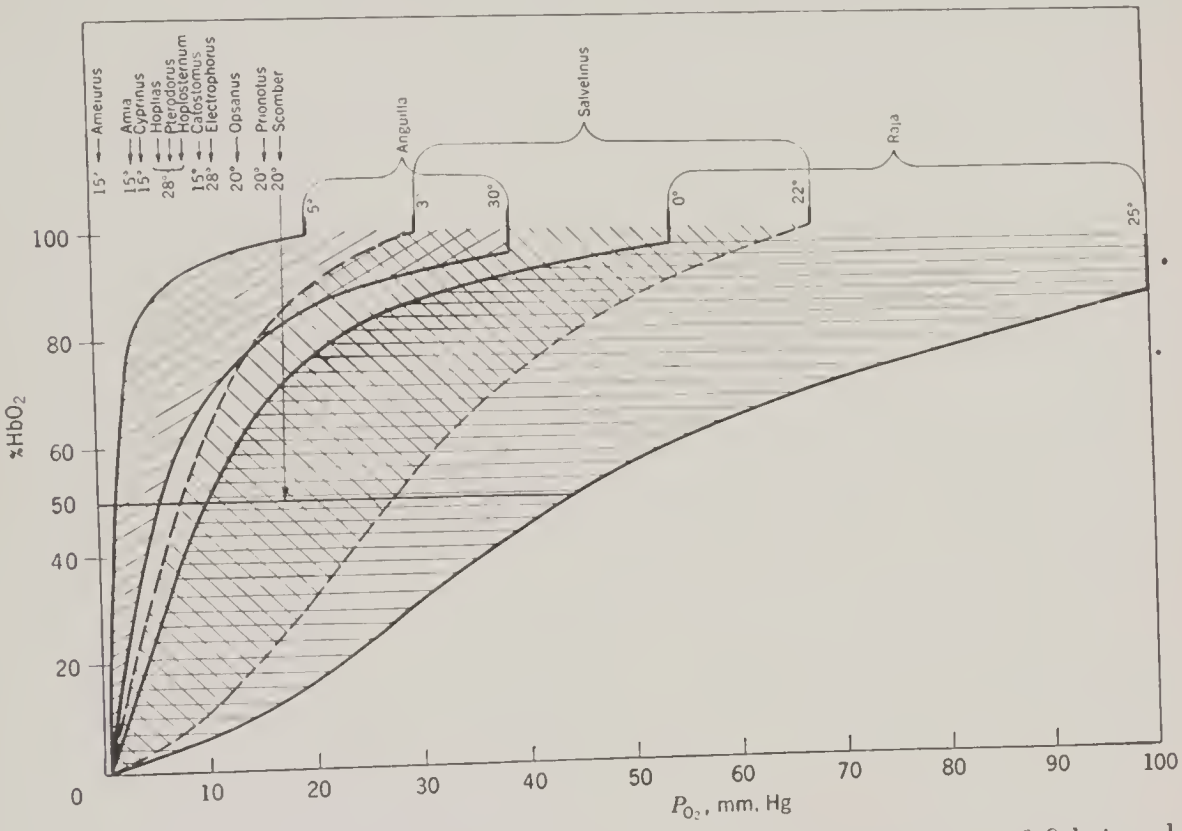


Fig. 5. Oxygen dissociation curves for the bloods of three species of fish in relation to temperature and oxygen pressure together with the positions of the half-saturation points of the bloods of various other species determined at single temperatures. Data from the following sources: *Anguilla japonica* (Kawamoto, 1929); *Salvelinus fontinalis* (Irving *et al.*, 1941); *Raja diaphenes* (Dill *et al.*, 1932); *Ameiurus nebulosus, Amia calva, Cyprinus carpio, Catostomus commersonii* (Black, 1940); *Hoplias malabaricus, Pterodorus granulosus, Hoplosternum littorale, Electrophorus electricus* (Willmer, 1934); *Opsanus tau, Prionotus carolinus, Scomber scombrus* (Root, 1931).

samples must be used within a few hours of being drawn. Care in handling the fish themselves is also extremely important as is indicated by the recent work of Black (1955) who demonstrated increase of lactic acid in fish unless extreme care is taken to prevent them from being excited or struggling. The marked effect of lactic acid on the behavior of fish blood (Auvernat and Secondat, 1942; Black and Irving, 1938; von Buddenbrock, 1936; Redfield, 1933; Secondat and Diaz, 1942) is

well established. A technique in handling worth investigation is the use of electric shock as an anesthetic (see Lindroth, 1952).

Oxygen dissociation curves for fish blood are shown in Fig. 5. Here the extremes found for dissociation curves over a range of temperatures are given for three species to show the magnitude of the temperature effect as compared with the specific differences which are also displayed. The effect of increasing temperatures is to increase the partial pressure of oxygen required to saturate the blood. The magnitude of this shift

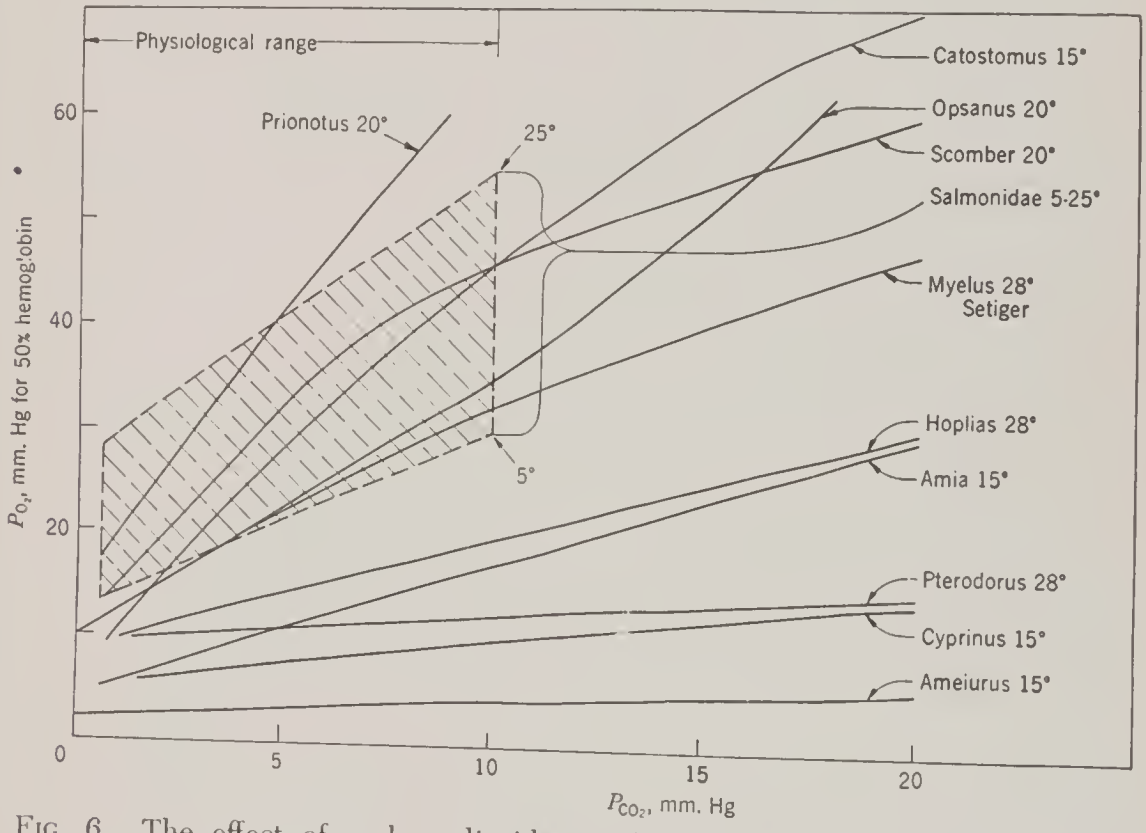


FIG. 6. The effect of carbon dioxide on the uptake of oxygen by fish blood. Data from the same sources as in Fig. 5 except Kawamoto.

is considerable, especially in the case of *Raja*, although each species has its characteristic position in the entire range. In addition (this is not shown in Fig. 5), there is a moderate reduction in the absolute oxygen capacity with increasing temperature (about 15% decrease from 5° C. to 30° C. in *A. japonica* according to Kawamoto (1929)). Because of the magnitude of the temperature effect, no curves of species in which the blood has been investigated at a single temperature only have been plotted in Fig. 5. However the half-saturation points are indicated for various such determinations in the more recent literature.

A second important feature of the behavior of blood with respect to transport of the respiratory gases is the effect of increased carbon dioxide

on the retention of oxygen; this is shown in Fig. 6. Here again, as illustrated by the hatched area which represents the data of Irving *et al.* for three Salmonidae, temperature has a major effect on the course of the curve for the blood of a given species. However, there do not appear to be data available for further comparisons of this type so that the remainder of the data represent curves at single temperatures. These latter curves represent 3 marine (Root's data), 4 temperate freshwater

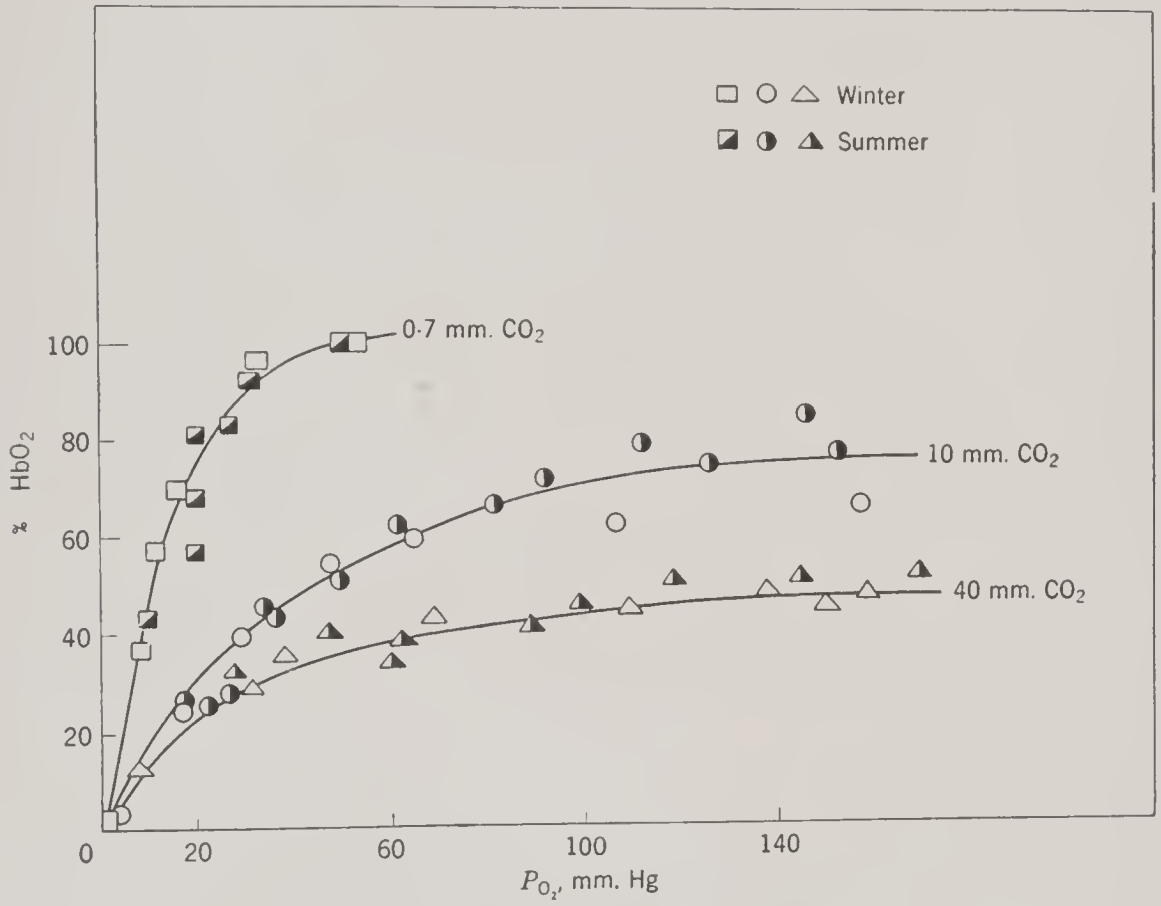


FIG. 7. Comparison of the blood of *Salvelinus fontinalis* drawn from fish acclimated to winter or to summer conditions. All samples equilibrated at 15° C. Unpublished data of E. C. Black.

(Black), and 3 tropical freshwater species (Willmer). The spread of the curves of these three groups is such that probably no region of the range displayed is typical of fish of any one of these habitats although the 3 marine examples all have blood sensitive to CO_2. Other acids of course also influence the loading of oxygen by fish blood in a fashion similar to carbon dioxide as in other bloods.

A characteristic of fish blood as opposed to the blood of terrestrial vertebrates (not displayed in Fig. 6) is that at higher levels of carbon dioxide the hemoglobin never becomes completely saturated with

oxygen. This effect of acid completely preventing part of the hemoglobin from combining with oxygen was first noted by Root (1931) and is often named after him the "Root" effect. In bloods of some species, e.g. *Epinephelus mystacinus* the Root effect has been observed in the presence of oxygen up to pressures of the order of 100 atmospheres (Scholander and van Dam, 1954).

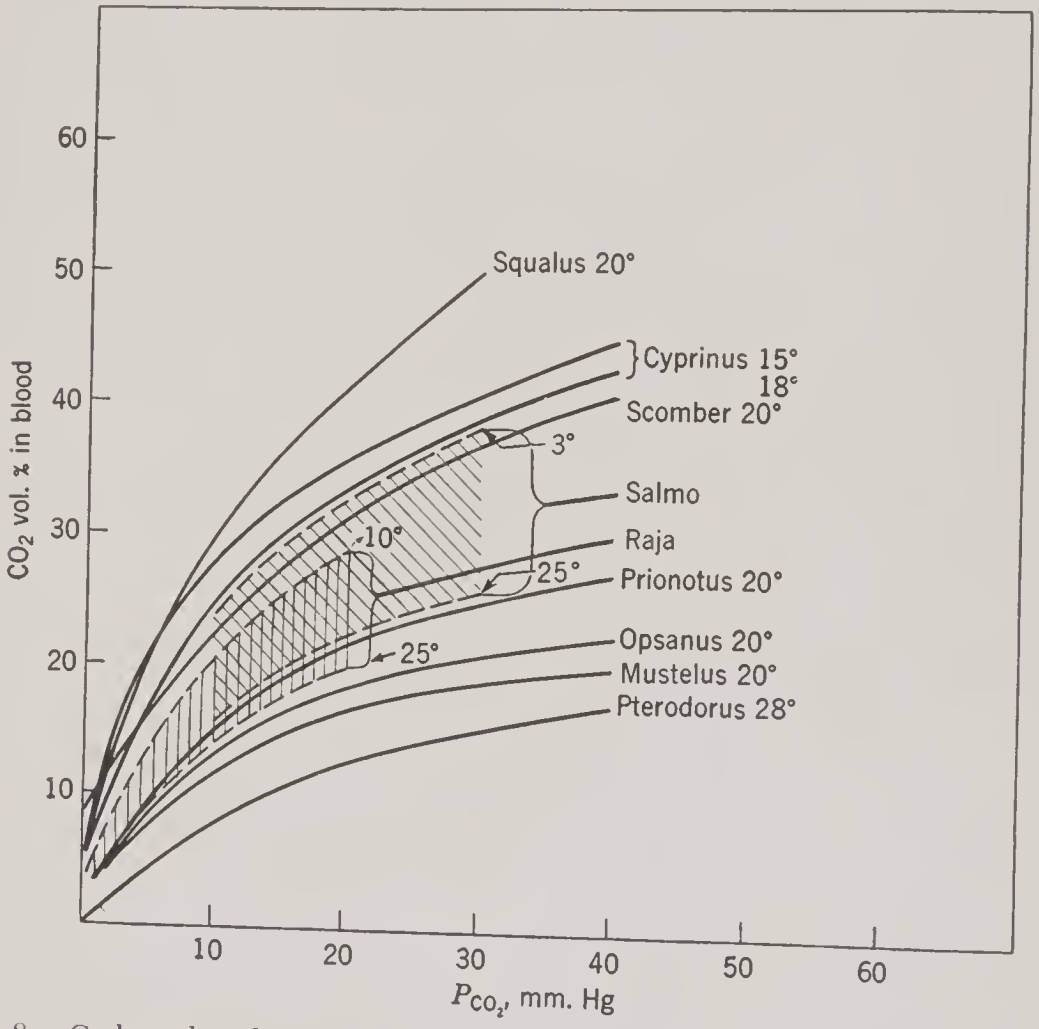


FIG. 8. Carbon dioxide dissociation curves for fish blood. Data from the following sources: *Squalus acanthias, Mustelus canis* (Ferguson *et al.*, 1938); *Cyprinus carpio, Salmo gairdnerii* (Ferguson and Black, 1941); *Scomber scombrus, Prionotus carolinus, Opsanus tau* (Root, 1931); *Raja diaphenes* (Dill *et al.*, 1932); *Pterodorus granulosus* (Willmer, 1934).

The variation of the oxygen dissociation curves with temperature is to be ascribed to a direct effect of temperature on the biochemical behavior of the blood and not to an homeostatic response on the part of the whole organism, which response would indeed be expected to go in the opposite direction. Black (1951) and Black and Black (1946) report no change in the respiratory characteristics of the blood of 2 species of

Salmonidae drawn from summer and winter fish but compared at the same temperature. Black's data for *Salvelinus fontinalis* are shown in Fig. 7.

2. Carbon Dioxide Transport

The characteristics of fish bloods with respect to the transport of carbon dioxide are shown in Fig. 8. Again temperature has a major influence on the position of the dissociation curve as is illustrated by the data for *Salmo gairdnerii* and *Raja diaphenes (R. ocellata)* the blood having a higher CO_2 capacity at a lower temperature. The curves shown are mean curves between reduced and oxygenated blood. In fish, as in the higher vertebrates, reduced blood has a higher carbon dioxide capacity than oxygenated blood (Root, 1931; Dill *et al.*, 1932; Root and Irving, 1941; Ferguson and Black, 1941). Sometimes however this effect cannot be detected (Willmer, 1934; Ferguson *et al.*, 1938). Fish (Root, 1931) have bloods of lesser buffering power than mammals but this deficiency is not so great at low partial pressures of carbon dioxide (Ferguson and Black, 1941).

E. C. Black (personal communication) has shown that the average effect of exercise to the point of exhaustion is to lower the pH of the venous blood by about half a unit compared with the unexercised state (*Salmo gairdnerii,* unexercised 7.41, exercised 7.15; *Cyprinus carpio,* unexercised 7.86, exercised 7.45; *Ameiurus melas,* unexercised 7.91, exercised 7.57; all at approximately 11° C.). The total range of pH reported for fish bloods is from approximately 6 (Willmer, 1934, tropical fish) to slightly under 8. The extreme range reported for one species appears to be the data of Hall and Gray quoted by Barcroft (1934) for *Stenotomus chrysops* in which various samples of venous blood gave pH values from 6.60 to 7.52. Powers, Rostorfer, and Rostorfer (1939) give a series of observations on the venous blood of some freshwater fish.

3. Estimates of Normal Blood Gas Levels

To interpret the data derived from blood curves it is necessary to have an estimate of the partial pressures of oxygen and carbon dioxide at the respiratory surface and in the tissues. Blood leaving the gills, because of the countercurrent flow of the ambient and the transport mediums, is as nearly in equilibrium with the respiratory gases in the water as the pumping capacity of the respiratory system and the rate of diffusion will permit. Under most circumstances, therefore, fish blood will take on its final oxygen in the presence of very low pressures of carbon dioxide and relatively high pressures of oxygen. The partial pressures of carbon dioxide which have been obtained for arterial blood of fish are

low. Root (1931) estimated a p_{CO_2} of 2 mm. Hg for blood taken from the gills of *Prionotus carolinus* and Dill *et al.* (1932) estimated a p_{CO_2} of 1.4 mm. Hg for efferent blood from the gills of the skate. Ferguson and Black (1941) estimated the arterial p_{CO_2} in 4 freshwater species to be 3–5 mm. Hg. The partial pressures of carbon dioxide in the venous blood of fishes are also correspondingly low, being 10 mm. Hg and less in fish from well-aerated water [*Opsanus tau,* 10 mm. Hg (Root, 1931); *Salmo gairdnerii, Cyprinus carpio, Ameriurus nebulosus,* 5–10 mm. Hg (Ferguson and Black, 1941); *Salmo salar,* 6–8 mm. Hg summer, 3–6 mm. Hg winter (Black and Black, 1946)]. Oxygen saturations for venous and arterial blood are given in Table VI, Chapter II (p. 94).

The high CO_2 effect on the oxygen-holding ability of the blood shown in Fig. 6 often aids in the shift of oxygen to the tissues as Krogh and Leitch (1919) and Black (1940) in particular have pointed out. The rapid rise of the dissociation curves for carbon dioxide over the probable physiological range (partial pressures from 0 to 20 mm. Hg) indicates that the shift of carbon dioxide is probably quite equal to that required by the oxygen capacity, particularly in those species where there is an appreciable shift in CO_2 capacity at the gills occasioned by the oxygenation of the hemoglobin.

D. Summary

The respiratory system of the water-breathing fishes is well adapted to the environment. The flow of the dense medium is unidirectional whenever circumstances allow. The disposition of the respiratory surfaces and the course of the blood make for a high efficiency of interchange of the respiratory gases. Ventilation may be a by-product of locomotion in pelagic fish. The blood as a transporter of respiratory gases has characteristics which operate well over the range of temperature and gas concentrations which fishes encounter.

An interesting aspect of the respiratory system in fish is the great range of quantitative variation found in the components of a system which qualitatively is exceedingly uniform throughout the class. There is a great, though not yet quantitatively expressed, range in the development of the branchial pump. The respiratory surfaces (Table II) show at least a ten-fold variation. There are equally great variations in the quantitative aspects of the components of the blood involved in the chemical transport of the respiratory gases. The mechanical aspects of the circulatory system, the blood volume, the blood pressure, and the stroke output (Chap. II) also vary to a significant degree from species to species.

This great range of quantitative variation leads to a wide diversity

of response as is indicated in Fig. 9 which displays the degree to which various species can remove oxygen from water in the presence of various concentrations of carbon dioxide when sealed in bottles of limited size.

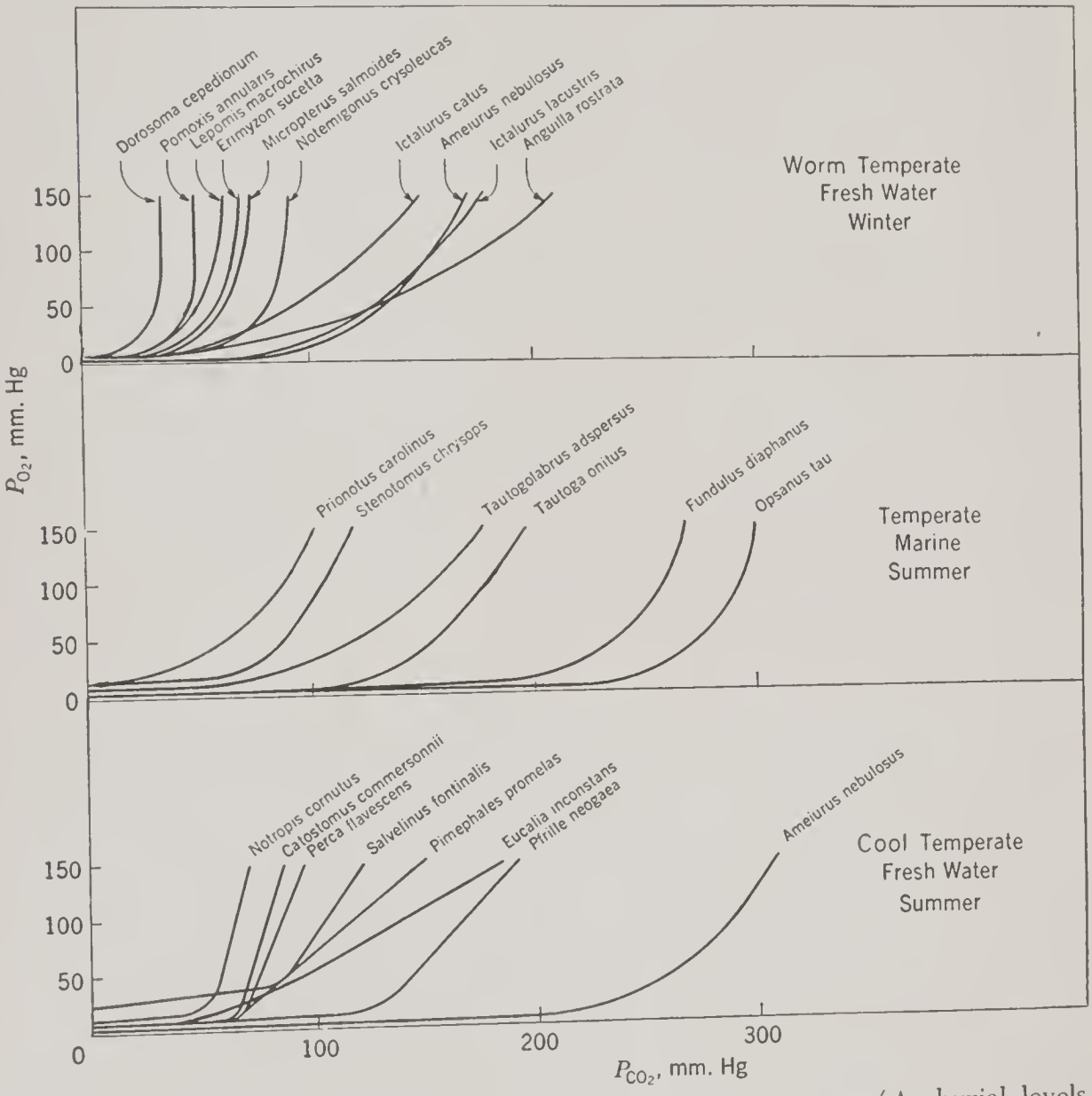


Fig. 9. Diversity in the response of fish to respiratory stress. (Asphyxial levels of oxygen at different carbon dioxide tensions.) The difference between the warm temperate and cool temperate forms shown in the figure is a seasonal not a geographic one. Note the position of *Ameiurus nebulosus* in the upper and lower panels. The spread between these two curves represents the approximate range of seasonal variation. Data for upper panel from Hart (1945); middle panel Safford (1940); lower panel Black *et al.* (1954).

Certain correlations are to be observed in the variation of the various components of the respiratory system; these apparently tend to produce organisms each able to respire at somewhat the same level under different

circumstances. Thus in teleosts, Gray's observations on the area of the respiratory surface place the species in an order inverse to the development of the branchial pump as indicated by Baglioni's classification. The pelagic species characterized by a much reduced branchiostegial apparatus and those which, as exemplified by the mackerel (Hall, 1930), depend on forward motion to irrigate their gills, present a large, finely divided respiratory surface to be ventilated perhaps by a relatively slow passage of water. The demersal fish on the other hand have smaller gill areas but have these disposed about wider, longer tubules which are vigorously irrigated by the branchiostegial pump. Hart (1943) showed an inverse correlation between the stroke output of the heart and the magnitude of the Bohr effect on the blood in 4 freshwater species and (Hart, 1945) with the sensitivity towards carbon dioxide (Fig. 9) in an additional series of 6 species.

In addition to the correlation of the ventilatory mechanisms with the habit (Baglioni) and that of the extent of the respiratory surface with activity (Gray) mentioned above, certain correlations with the environment are demonstrable. Krogh and Leitch (1919) pointed out early the advantage of a high Bohr effect to species which were active in cold water. Black (1940) extended this correlation. Willmer (1934) showed a correspondence between the magnitude of the Bohr effect and the gradient of oxygen and carbon dioxide in tropical fresh waters. There is, as far as the data are available, one interesting lack of correlation. There is no relation at all between the lethal temperature of fish and their respiratory sensitivity as indicated in Fig. 9. Species for which this comparison can be made are listed in Table III.

III. THE METABOLIC RATE

The metabolic rate, properly speaking should be measured in terms of calorific output. This can be done most conveniently by indirect calorimetry. A complete investigation of a species from this point of view would be very valuable but such a study has been attempted only rarely and never apparently been carried out completely. Such an investigation requires the determination of the oxygen intake, carbon dioxide output, and nitrogen excretion together with an estimate of the calorific value of the excreta. Practically all the work on indirect calorimetry was carried out early and the best collected account of this is to be found in Wunder (1936).

Ordinarily the rate of oxygen consumption alone has been taken as a general measure of the intensity of metabolism. Such measures have been made to determine the ability of a given species to take up oxygen

when under the influence of various external factors including gradations of the partial pressure of oxygen itself; this is indeed one of the most important of the identities to fluctuate in the aquatic environment. Measurements of the metabolic rate for this second purpose require consideration of two rates of metabolism, a minimum rate which is the minimum consistent with the continued existence of the animal and a maximum rate which is the level which will permit the highest continued level of

TABLE III

Relation between Respiratory Sensitivity as Shown in Fig. 9 and the Upper Lethal Temperature [a]

Species	Approximate Upper Lethal Temperature ° C.
High Respiratory Sensitivity	
Dorosoma cepedianum	36
Notropis cornutus	32
Catostomus commersonnii	30
Perca flavescens	33
Lepomis macrochirus	34
Moderate Respiratory Sensitivity	
Salvelinus fontinalis	25
Pimephales promelas	34
Micropterus (Huro) salmoides	35
Notemigonus crysoleucas	35
Low Respiratory Sensitivity	
Pfrille neogoea	32
Ameiurus nebulosus	36
Ictalurus punctatus	34

[a] The upper lethal temperature given is the highest temperature at which the species can live after extended acclimation, the *ultimate upper lethal temperature* of the authors cited. References as in Fig. 9 plus Brett (1944) and Hart (1947, 1952).

activity. In the discussion below these two rates will be termed respectively the standard and active rates of oxygen uptake.

An interesting point in connection with the oxygen consumption of fish is that the active rate of oxygen uptake is restricted to a few multiples of the standard rate. For example in the data shown in Fig. 24 (p. 50) the greatest augmentations of the active rates are only of the order of four times the standard rates. In contrast (Krogh, 1941) the increase with maximum work in man is twenty-fold and in insects it may be of the order of a hundred-fold. The consequence of this restriction of the maximum rate in fish is that the respiratory surface rather than the

absorptive surface of the digestive tract may be the limit of the maximum rate of growth of which fish are capable. Job (1954) in a single preliminary experiment found that the consumption of minnows by two yearling *S. fontinalis* fed to repletion required a rate of oxygen consumption for the assimilation of this food equivalent to their active oxygen consumption as determined in the rotating chamber. Due allowance was taken in this work for increase in weight and for the calorific content of the feces and urine (see also Chap. IX).

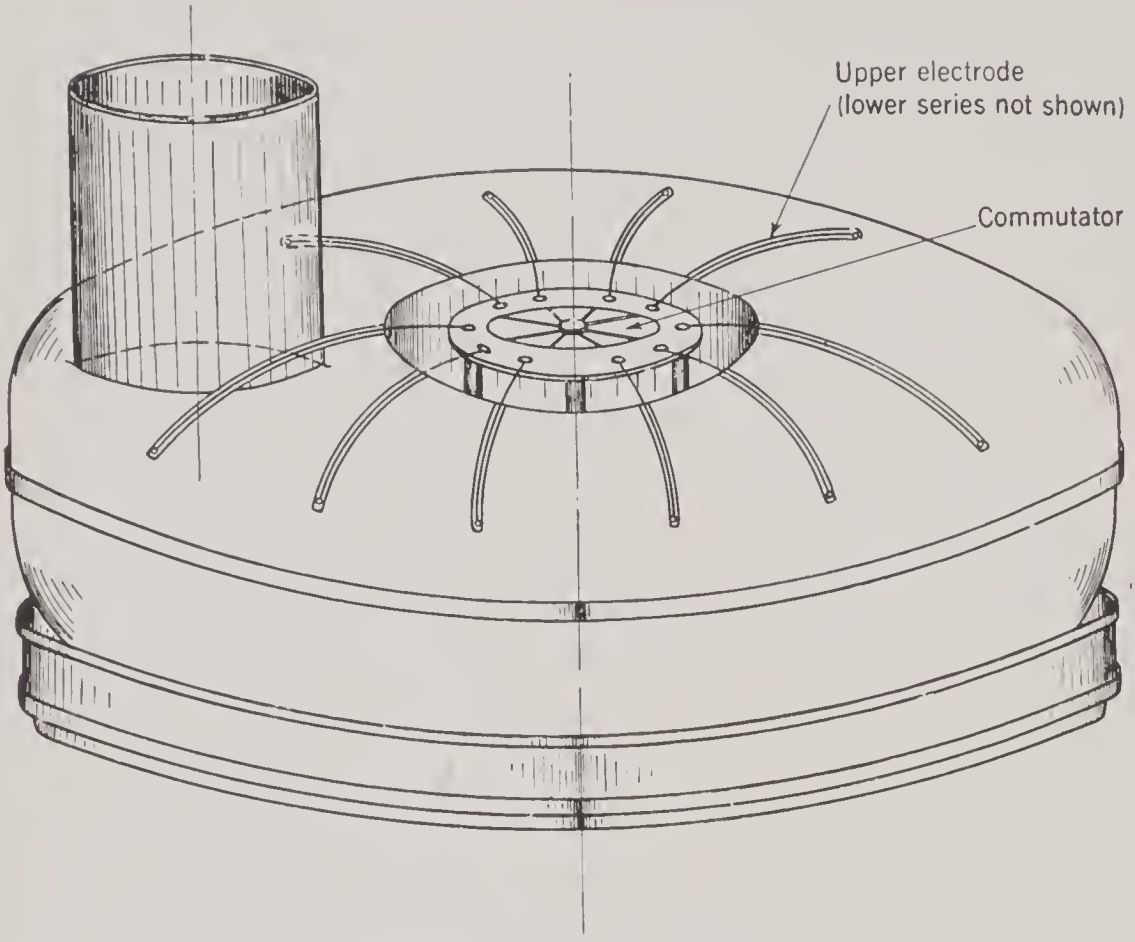
A. Methods of Measuring Oxygen Consumption

Three general methods have been used in determining the rate of oxygen consumption. These are: (1) following the depletion of oxygen in a sealed container, (2) measuring the loss of oxygen and the rate of flow of water through a small chamber, (3) manometric methods.

1. Apparatus

When the metabolic rate has been measured by the consumption of dissolved oxygen in a closed chamber the technique of experimentation may have been so simple as to involve merely placing the fish in a sealed container of water and finding the difference between the oxygen content of an initial sample and that of a second sample withdrawn at some later time. From the analytical point of view, the major objection to this technique is only that there is danger of stratification of the content of dissolved oxygen in the relatively large volume of still water in the chamber. Such stratification may make the oxygen content of the samples drawn for analysis erratic (Keys, 1930).

The simple closed chamber has been used in determining the respiration of fish under activity. The present apparatus used in the author's laboratory is shown in Fig. 10. The chamber is a torus moulded from transparent plastic with an opening on the upper side to a short vertical open cylinder. The chamber is provided with a series of pairs of aluminum electrodes mounted radially in the upper and lower sides, each electrode being approximately a quadrant. The lower series of electrodes are connected in a common circuit. The upper series are individually connected to segments of a commutator. In use the chamber is filled with a known volume of water to a point somewhat above the base of the vertical cylinder, the fish is introduced and paraffin poured on the surface of the water in the open neck to provide a seal. The chamber is mounted on a turntable and as it rotates the fish swims against the current of water. A pair of brushes transmit a low voltage alternating electric current to the commutator (3 volts, 60 cycles, for a chamber approxi-



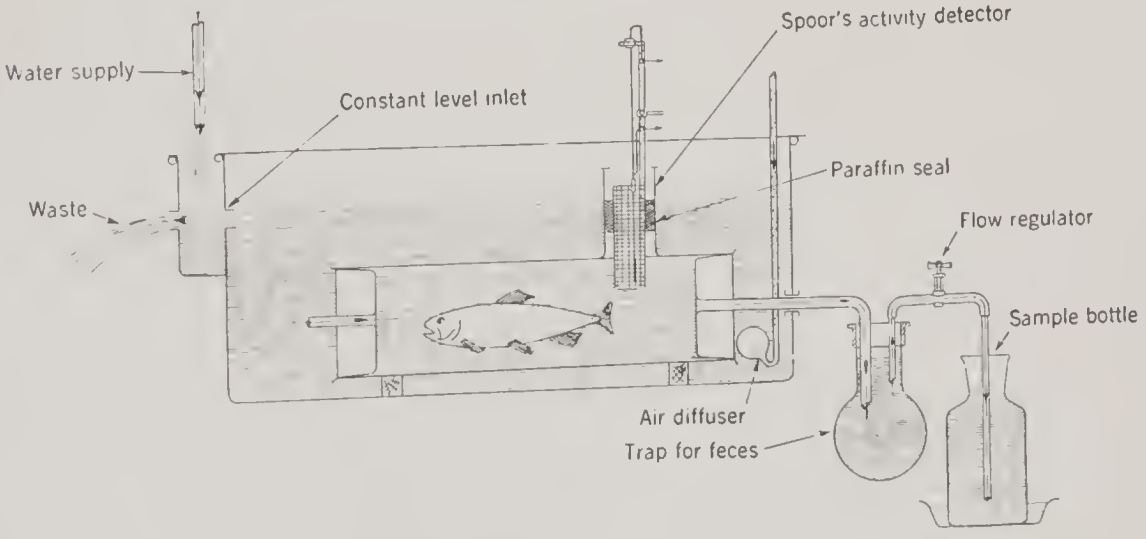
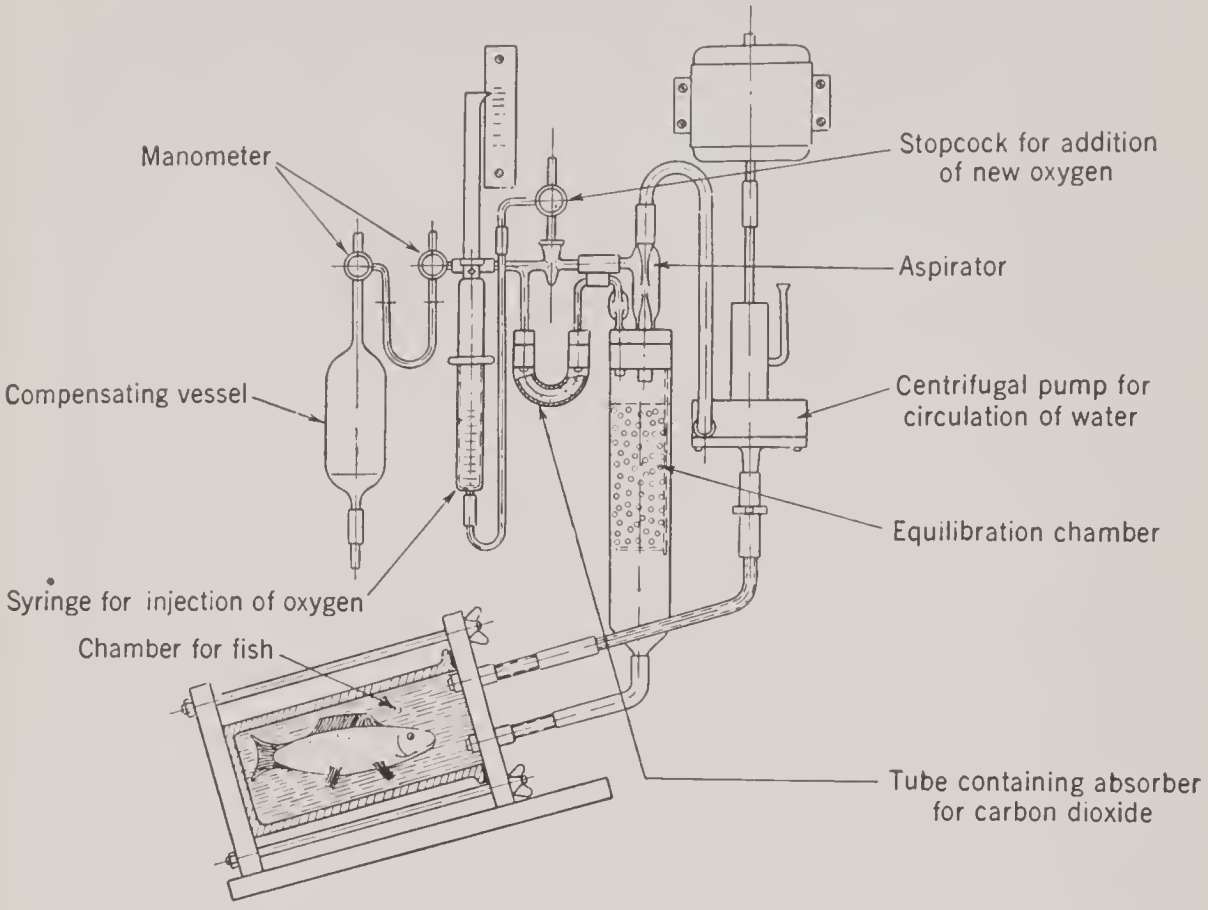
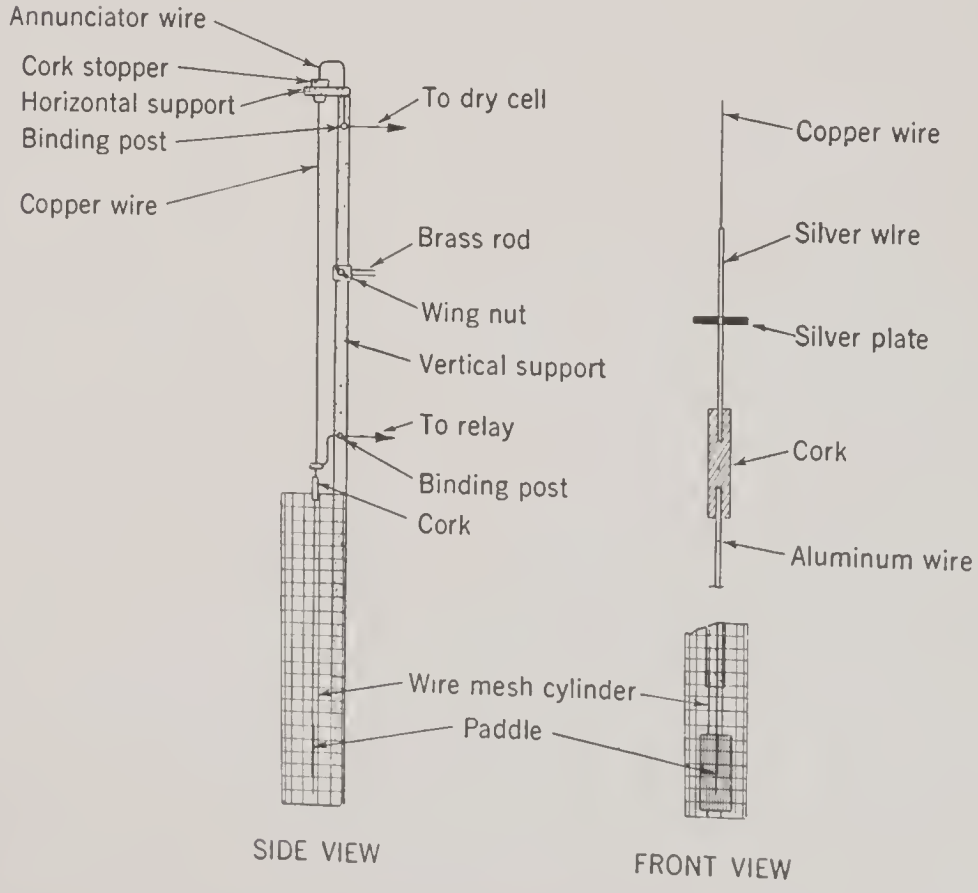


FIG. 10. Types of apparatus used in measuring the metabolic rate in fish. A, closed rotating chamber used in determining the active metabolic rate; B, constant flow system incorporating Spoor's activity detector (for standard metabolism); C, gasometric system (Scholander *et al.*, 1943); D, Spoor's (1941) activity detector.


Manometer
Stopcock for addition of new oxygen
Aspirator
Compensating vessel
Centrifugal pump for circulation of water
Equilibration chamber
Syringe for injection of oxygen
Chamber for fish
Tube containing absorber for carbon dioxide


Annunciator wire
Cork stopper
Horizontal support
Binding post
Copper wire
To dry cell
Brass rod
Wing nut
Vertical support
To relay
Binding post
Cork
Wire mesh cylinder
Paddle
SIDE VIEW
Copper wire
Silver wire
Silver plate
Cork
Aluminum wire
FRONT VIEW

mately 5 inches in sectional diameter, water K 18 $\times$ 10^6, 311). Thus the electrodes are charged momentarily in succession as they pass the point where the brushes are placed. Since the lower set of electrodes are interconnected, a fringe of this electric field extends well beyond the point of greatest intensity. If the fish fails to breast the current of the rotating water in the chamber and begins to fall back, it is ordinarily stimulated by the electric field and renews its efforts so that by choice of an appropriate speed of rotation the fish may be maintained in a continuously active condition. A light is fixed near the chamber just in advance of the position where the electrodes become charged to serve to identify the charged segment for the fish. The chamber is stopped to take samples of the water and an equal volume of water of known oxygen content is introduced whenever a sample is withdrawn for analysis, thus the volume of water in the chamber is kept constant. The apparatus described here is a modification of that figured by Fry and Hart (1948b).

When measuring respiration by the estimation of dissolved oxygen, the more widely used experimental technique has been to place the fish in a chamber through which water flows slowly and to measure oxygen consumption by analyzing the inflowing and outflowing water and the rate of water flow. A typical arrangement of apparatus (except for the suggested addition of the activity detector) for this method is also illustrated in Fig. 10. The bath acts as a constant head of aerated water which flows through the chamber and out to the sample bottle, the flow being regulated by a clamp. The rate of flow is measured by collecting the overflow from the sample bottle. A more refined apparatus of this type is illustrated by Wells (1935a) and similar arrangements are figured widely in the literature.

One of the great advantages of the gasometric technique is that it can be readily adapted to a wide scale of rates of oxygen consumption. It is particularly suitable for measuring the respiration of very small organisms such as a single fish egg or embryo. For this purpose the Cartesian diver method (Zeuthen, 1947) or Scholander's (1942) micro-respirometers are available. The second advantage of the gasometric method is the relative ease with which it can be adapted for automatic recording. This is ordinarily done by replenishing the oxygen from a reservoir as it is consumed (Scholander, Haugaard, and Irving, 1943). Brown (1954) has recently employed a flexible sac as the oxygen reservoir. This sac together with the remainder of the apparatus, which serves to weight the sac down as well as to accommodate the organism, is suspended in a constant-temperature water bath from an appropriate balance. Consumption of 1 ml. O_2 from the sac records 1 g. on the

balance. Brown found Saran tubing (Visking Corporation Chicago) the most impermeable material for the sac.

When metabolic studies are being carried out for the purposes of indirect calorimetry, the manometric method is to be preferred since it permits the collection of the nitrogenous end-products. A typical application of the manometric method is illustrated in Fig. 10. Another

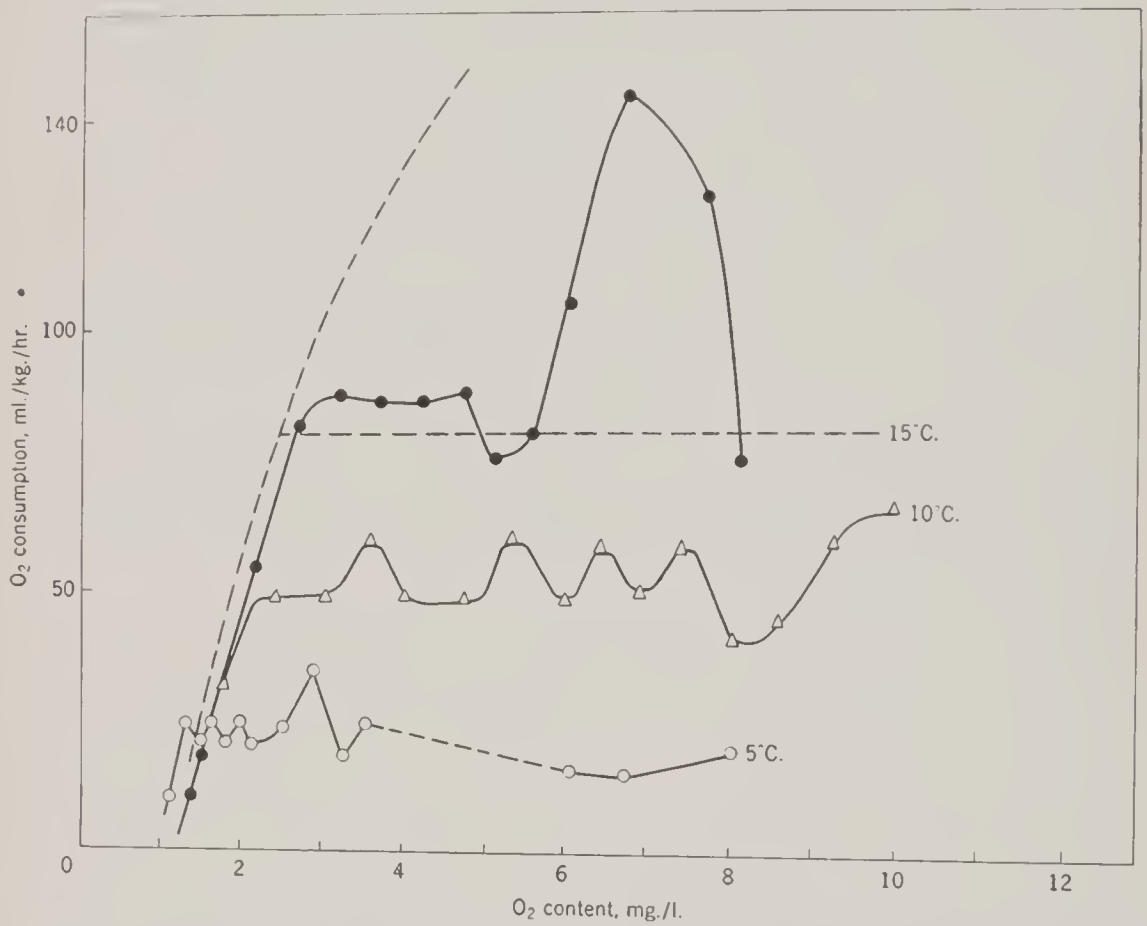


FIG. 11. The resting metabolism of the goldeye, *Amphiodon alosoides*, in relation to temperature and oxygen concentration. Unpublished data of J. S. Hart, courtesy the Fisheries Research Board of Canada. The horizontal line through the 15° C. points is the estimated standard metabolic rate. The inclined broken line it meets is the active metabolic rate (see Fig. 14). The time sequence reads from right to left.

convenient application of the manometric method is in the determination of the respiration of fish eggs *en masse*. Eggs can be maintained in moist air and treated essentially as terrestrial organisms (Amberson and Armstrong, 1933). Leiner (1937) also found the manometric method most suitable for his work on the seahorse. Use of respiration apparatus where the chamber itself is continuously agitated cannot be recommended for the measurement of standard metabolism since it disturbs the organisms. Similarly the device to mix the water and air should be

designed to disperse thoroughly a small amount of water rather than to agitate vigorously the whole volume in the chamber.

In all apparatus for measuring respiration an important point to be considered is the well-known permeability of rubber tubing to oxygen.

2. Precautions to be Observed in the Measurement of the Metabolic Rate

To introduce this subject a brief consideration of the results obtained in measuring the metabolic rate is given at this point. Figure 11 illustrates some unpublished results obtained by J. S. Hart for the goldeye, *Amphiodon alosoides*. Hart's experiments were carried out in a very large chamber which had sufficient volume to provide the fish with oxygen for two or three days. His results are typical of the way the respiration of fish is influenced by activity and by oxygen content. Take for example the measurements of the respiratory rate presented in Fig. 11 for 15° C. Here during the early part of the experiment the rate of oxygen uptake rose considerably in response to the activity of the fish; later it settled down to a steady value which remained constant until the oxygen in the chamber had been depleted to a content of 2.5 mg./l. At lower levels of oxygen the rate of respiration was progressively depressed.

The characteristics of Hart's data typify the problems of the experimenter who wishes to measure the metabolic rate of fish. Over a certain range of oxygen pressures (and the complementary pressures of respired carbon dioxide), the routine metabolic rate of the fish is independent of the oxygen pressure but can fluctuate widely under the influence of activity or excitement. Black, Fry, and Scott (1939) report for example that the handling of fish required to place them in the respiration chamber is sufficient stimulus to cause them to consume oxygen at their maximum rate for some time afterward. Keys (1930), Wells (1932) and Ivlev (1938) give typical series of readings in respiration chambers covering the first hours after introduction of the fish showing the high initial rates which only gradually subside. An example of the relation of activity to oxygen consumption is given in Fig. 12. It is further necessary to pay attention to the size of the chamber in relation to the size of the fish. A chamber which is unduly small tends to overexcite the fish. Geyer and Mann (1939b) report for *Perca fluviatilis* that the chamber should have a volume at least ten times that of the fish.

The measurement of standard metabolism, if the worker wishes to approach basal conditions, thus requires a delay after the introduction of the fish before measurements begin. It requires also the suppression of external stimuli that will excite the fish to activity. Usually this is

accomplished by the maintenance of darkness and quiet. Fish, however, often show endogenous daily cycles of activity so that the level of metabolism may depend on the time of day in spite of the maintenance of constant conditions. Figure 13 shows a daily cycle of oxygen uptake under conditions designed to display the standard metabolic rate when the subjects were kept in constant darkness.

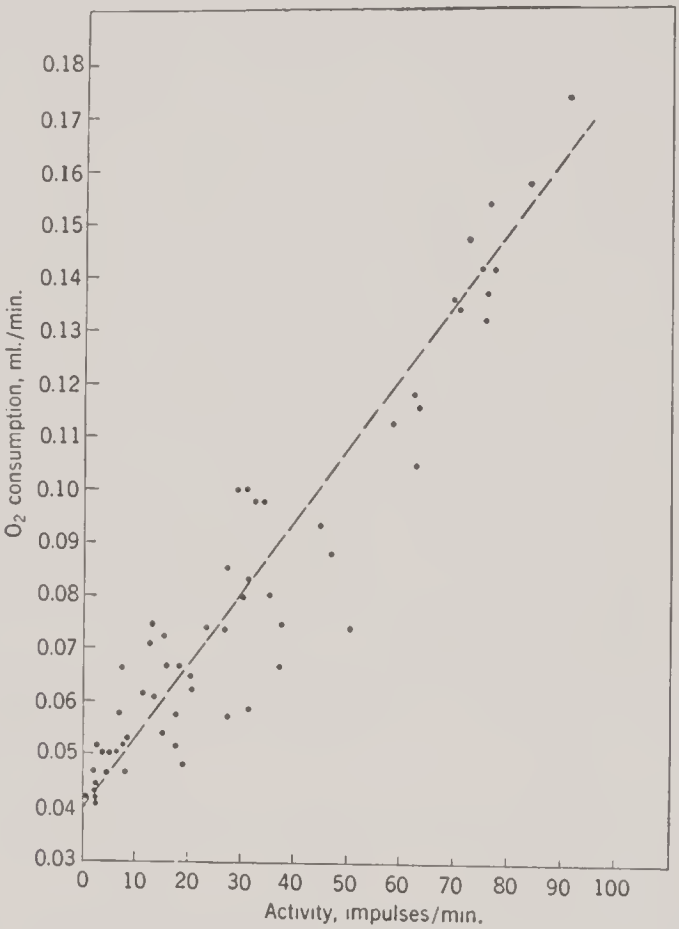


FIG. 12. The relation of oxygen consumption in the goldfish, *Carassius*, to activity, 32 g. subject, 23–25° C. The activity scale represents the number of impulses on Spoor's (1941) activity detector (from Spoor, 1946).

The endogenous cycles of fish are variable in their strength and timing even within one species (e.g. Spoor, 1946), and between species there is a wide difference in behavior in this respect. The yellow perch, *Perca flavescens*, for example becomes quiescent almost immediately after it is placed in a dark chamber (Ferguson, personal communication). Many species are of course nocturnal (e.g. Higginbotham, 1947) and darkening the chamber would not bring about quiescence. Others are crepuscular, e.g. Spoor and Schloemer (1939), Oliphan (1940), and the example shown in Fig. 13. It seems necessary that any series of measurements from which a measurement of the standard metabolism is desired should be carried out over at least one 24-hour period no

matter how constant the experimental situation is maintained. It is often advantageous to ensure further the approach to basal conditions by taking advantage of any reactions of the fish known to be conducive of quiescence. Thus the brown bullhead, *Ameiurus nebulosus,* is markedly thigmotactic and it will remain almost motionless if a small kennel consisting of an inverted "U" channel of appropriate dimensions is provided for it. Other species will be quieter when in groups, for example the goldfish (Schlaifer, 1939) and the speckled trout (Job, 1955). The explanation of Geyer and Mann (1939a) for reduction in the respiratory rate for groups appears to relate to active metabolism, i.e. their tests must have been over short periods immediately after transfer of the fish.

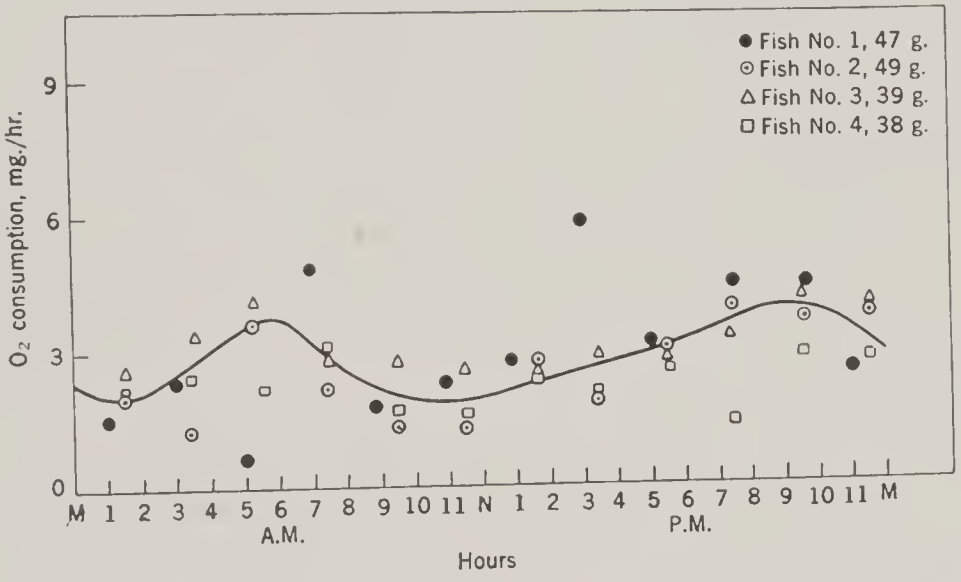


FIG. 13. An endogenous cycle in the standard metabolic rate; *Micropterus salmoides* at approximately 18° C. (from Clausen 1936).

Spoor's (1941) method of recording the activity of fish (see Fig. 10D) which he (1946) added to a respiration chamber should be considered seriously by all workers wishing to measure standard metabolism.

Certain workers (e.g. Ege and Krogh, 1914) have narcotized their subjects but this does not appear to be entirely satisfactory. Schlieper (1952) employed Dijkgraaf's (1949) method of immobolizing fish by severing the spinal cord between the 6th and 7th vertebrae in his measurements of standard metabolism.

The previous thermal history of the fish has a major influence on its metabolic rate. Three effects of the thermal history have been recognized. In general (e.g. Scherbakov, 1937; Sumner and Doudoroff, 1938; Haugaard and Irving, 1943) the standard rate will be higher at a given temperature if the animal has been previously maintained at a somewhat lower one and vice versa, but there are limits to this generalization.

If the change is too extreme, metabolism may be depressed (Christophersen and Precht, 1952) and indeed the animals may die. A decrease in temperature may have a temporary stimulating effect as Schlieper (1950) found for *Xiphophorus helleri* where for a period of many hours the metabolic rate of fish acclimated to 25° C. and transferred abruptly to 15° C. remained at a level much higher than that of fish acclimated to the latter temperature. This behavior may be presumably regarded as a reflection of increased physical activity associated with the mechanism of temperature selection (orthokinesis, Fraenkel and Gunn, 1940) and would be expected to occur at temperatures below the thermal preferendum. Sullivan (1954) has found the frequency of spontaneous movements of *Salvelinus fontinalis* to be greater at temperatures both above and below the thermal preferendum when the change is relatively rapid. Further reference to the subject of thermal stimulation will be found on p. 48.

Season as well as temperature may have an effect on the standard metabolic rate but this has not yet been well demonstrated although it is certain that differences in behavior between winter and summer are difficult to erase, e.g. Hart (1952), Sullivan and Fisher (1953), Flörke, Keiz, and Wangorsch (1954). Wells' (1935) study of *Fundulus parvipinnis* in which he found an increase in the metabolic rate in the spring in fish kept continuously at a constant temperature probably reflects an increase in diurnal activity attendant on the increase in day length as well as any change that might have taken place in the level of the standard metabolic rate.

Stabilization of the history of the experimental animal is therefore essential and preferably experiments should be carried out with reference to the annual cycle. Thus low-temperature experiments are best done in winter and those at high temperatures in summer.

If measurements of standard metabolism are being carried out, the oxygen content of the water leaving the respiratory chamber can ordinarily be dropped considerably below air saturation before the metabolic rate is affected. In order to set the safe limit to which the oxygen can be allowed to fall it is best to measure the relation of the active rate of oxygen uptake of the species in question, at least in a preliminary fashion prior to working on the standard metabolism.

Figure 14 shows Hart's data for the rate of respiration of the goldeye when active to an extent which presumably calls forth its maximum steady rate of oxygen consumption. These experiments were carried out in a rotating chamber (Fry and Hart, 1948b). Measurements of active metabolism can be carried out directly after the animal is placed in the chamber. It is only necessary to limit the length of the experiment to a

reasonable time to avoid the effects of fatigue. The active metabolic rate is ordinarily dependent on the level of oxygen in the water over a considerable range. Therefore measurements of active metabolism need to be made over a range of levels of oxygen concentration. It is often necessary to oxygenate the water in order to reach the maximum rate of oxygen uptake.

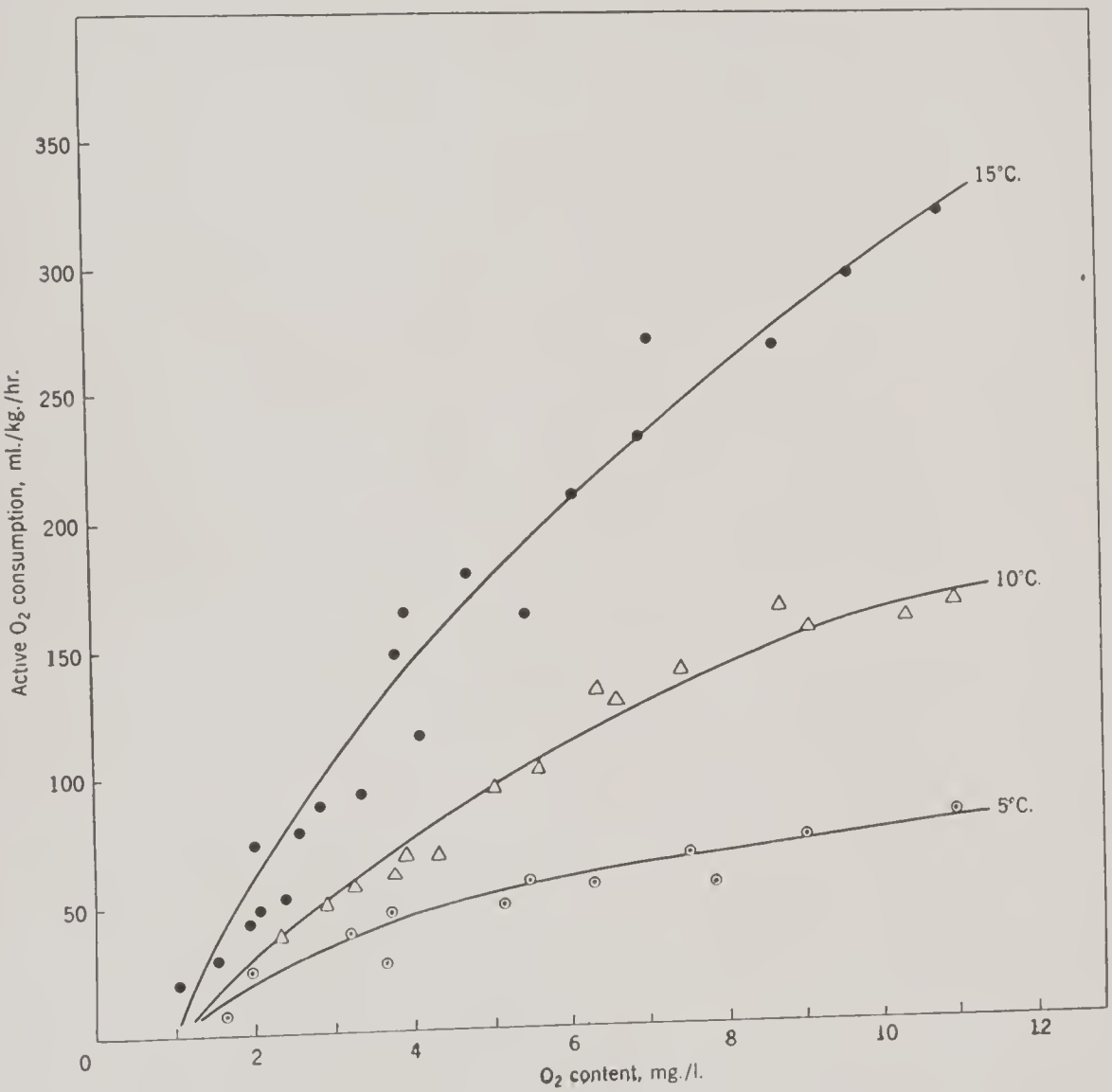


FIG. 14. Active metabolism of *Amphiodon alosoides* in relation to temperature and oxygen concentration. Unpublished data of J. S. Hart, courtesy of the Fisheries Research Board of Canada.

It is most important in connection with the measurements of active metabolism that the fish should be in good health. For example a dosage of chlorine in the water supply which makes it quite acceptable for domestic use will render it entirely unsuitable for the maintenance of fish from which one wishes to obtain a measure of active metabolism. Many parasites destroy areas of the gills so that disease often has a

permanent effect in reducing the active metabolic rate; the gills of fishes used in such experiments should always be examined.

The acclimation effects brought about by the thermal history may be as marked on the active rate as on the standard rate. The active rate of oxygen uptake at low levels of oxygen can also be influenced (Nikiphorov, 1953; Shepard, 1955) by the previous oxygen regime. It is increased by prior exposure to respiratory stress and results of ecological significance require that this fact be taken into account.

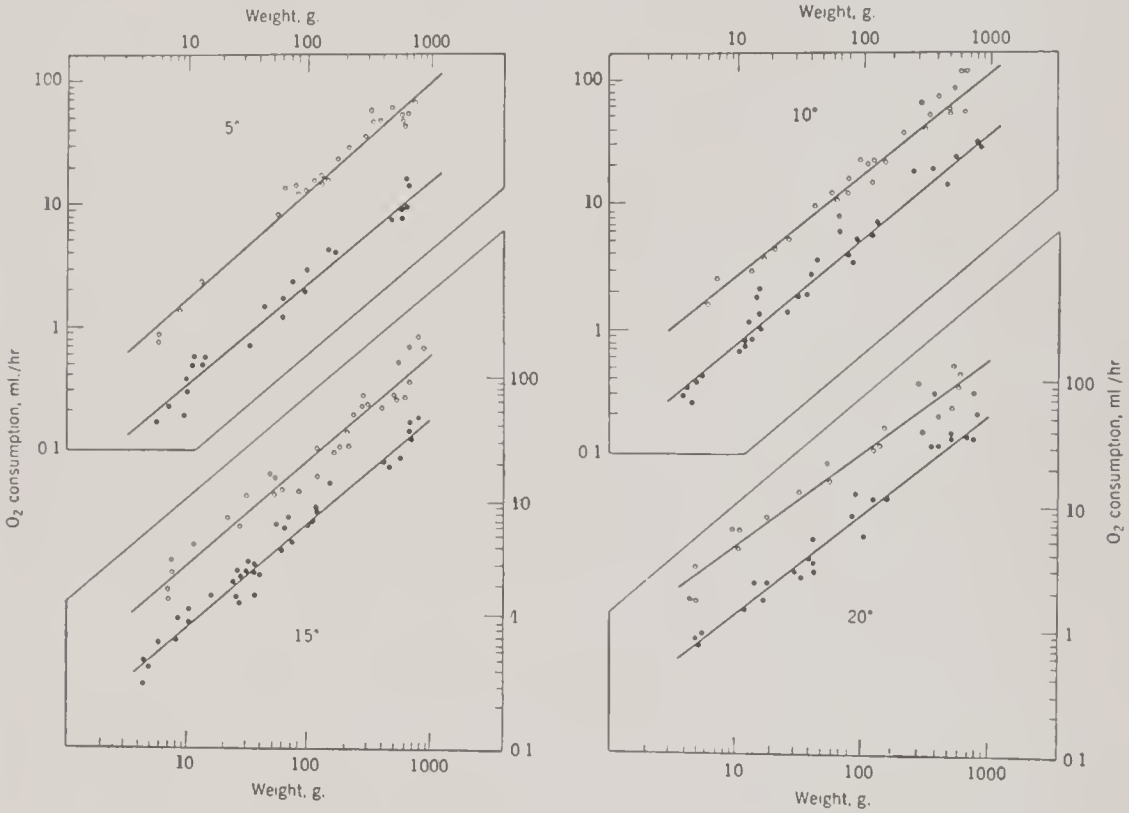


FIG. 15. Standard and active metabolism of *Salvelinus fontinalis* in relation to size and temperature (data of Job, 1955). Dots, standard metabolism; circles, active metabolism.

B. Metabolism in Relation to Size

The metabolic rate of fish in relation to size gives a straight line when the logarithm of the rate of respiration is plotted against the logarithm of the body weight. In the only species where it has been exhaustively investigated, the speckled trout, *Salvelinus fontinalis*, the proportional response of the standard metabolic rate to temperature is essentially independent of size (Job, 1955). Job's data are illustrated in Fig. 15. This appears to be a general phenomenon. The slope of the logarithmic line for standard metabolism for this species has a value of 0.85 which is intermediate between surface area dependence and weight dependence. Most species for which data are available have slopes relating the

logarithm of the rate of metabolism to the logarithm of weight that are of this order. Smith's (1935b) data for the African lungfish give a slope of approximately 0.9. Barrett (personal communication) obtained a slope of 0.8 for *Salmo gairdnerii* weighing from 5 to 75 g. The observations of Scholander *et al.* (1953) on both arctic and tropical fishes follow a general regression line for the logarithmic relationship under discussion of 0.85 (Job, 1955). Zeuthen's (1947) data for various species of fish give a slope of 0.78. Leiner (1937), Rubner (1924), and Morgulis (1914) among others also report deviations in the metabolic rate from the surface area law which requires a slope of ⅔ between the logarithms of rate and weight.

There are three species (two topminnows and one cyprinid) for which the data are at variance with this general finding. Keys (1931) and Wells (1935b) report data for *Fundulus parvipinnis* which give a slope to the log rate–log weight relationship of between 0.5 and 0.6, a very flat curve. However, Wells believed that in the data of both Keys and himself the values for the small fish are unduly high for various reasons and hence the curves should be steeper than the data show. Müller (1942) reports a slope of ⅔ for *Lebistes reticulatus*. The fragmentary data of Raffy and Fontaine (1930) for this species lend some support to Müller's findings. The data for *Cyprinus carpio* of Oya and Kimata (1938) also display a log weight–log oxygen uptake relationship with a slope of approximately two-thirds.

Job found the relation between the active metabolic rate and size for the speckled trout to be of the same general order as that for the standard rate. However, temperature has a slight but important effect on the relation of active rate to size. The slope becomes less with increasing temperature so that larger fish are at a greater disadvantage in warmer water. It is not known yet whether this is a general phenomenon for data are not yet available for more than the one species.

Since the relation of the logarithm of the metabolic rate to the logarithm of the body weight has a slope considerably less than unity in all cases investigated, the common practice of expressing the respiratory rate in terms of rate per unit weight must be recognized as a risky approximation and the weight of the experimental animals should always be given as well. Thus it is fortunate that Pereira (1924) gave the weight of his experimental animals when reporting the effect of pH on the respiratory rate. By replotting his data it is possible to see that the relation he reported is almost entirely to be ascribed to the influence of size. Flörke *et al.* (1954) recognized the possible inadequacy of the use of rate per unit weight in combining their results for fish of different sizes and used the ⅔ and ¾ power of the weight as well.

C. Metabolism in Relation to Starvation

Standard metabolism decreases markedly with starvation. Smith (1935a, b) who followed the respiration of the unfed lungfish, *Protopterus aethiopicus,* over periods to beyond 600 days at 25° C. has done the most thorough work on this subject. During the first 7 days the resting metabolism dropped to 50% of its initial value. At 300 days it was of the order of 15–20% of the initial value. The decline is essentially logarithmic (see Fig. 16) throughout at least the first 200–300 days with never any constant level. Feeding at any time immediately tends to restore the metabolism to the prestarvation rate. Raffy's (1933) experiments on the goldfish and young eels also display the same phenomenon well. van Dam (1938) observed a decrease in standard metabolism of the same order in an eel.

Barrett (personal communication) has investigated the effect of starvation on active metabolism in *Salmo gairdnerii.* His experiments which were carried out over 100 days at approximately 11° C. are also illustrated in Fig. 16. There was no essential difference in the ability of starved and control animals to consume oxygen when stimulated to perform work over this period. However, there was slight but significant tendency for the starved fish to require a higher oxygen pressure in the water to bring about their maximum rate of oxygen uptake.

D. Oxygen Consumption in Relation to Oxygen Content

Many workers have observed that the rate of uptake of oxygen by fish and many other organisms respiring in a closed vessel has decreased as the oxygen content of the water has fallen. The reviews of Tang (1933) and von Ledebur (1939) should be consulted for the earlier literature on this subject. So general is this phenomenon that it has received a special name "respiratory dependence." Although the observation was easily made there was much confusion concerning its meaning. Finally von Ledebur (1939) remarked that the observed restriction in oxygen consumption could not be interpreted as a restriction of vital processes. It is clear therefore that respiratory dependence should be investigated in relation to active metabolism; if organisms are to remain in oxygen balance, the restriction imposed on the respiratory rate by a decrease in the level of the ambient oxygen content means a restriction of the activity of the organisms and not of their strictly maintenance metabolism. There is no evidence for extensive anaerobiosis in fish. An illustration of the restriction on activity imposed by a lowered oxygen content is given in Fig. 17. Similar data for *Salvelinus fontinalis* have been published by Graham (1949). So many good cases of respiratory

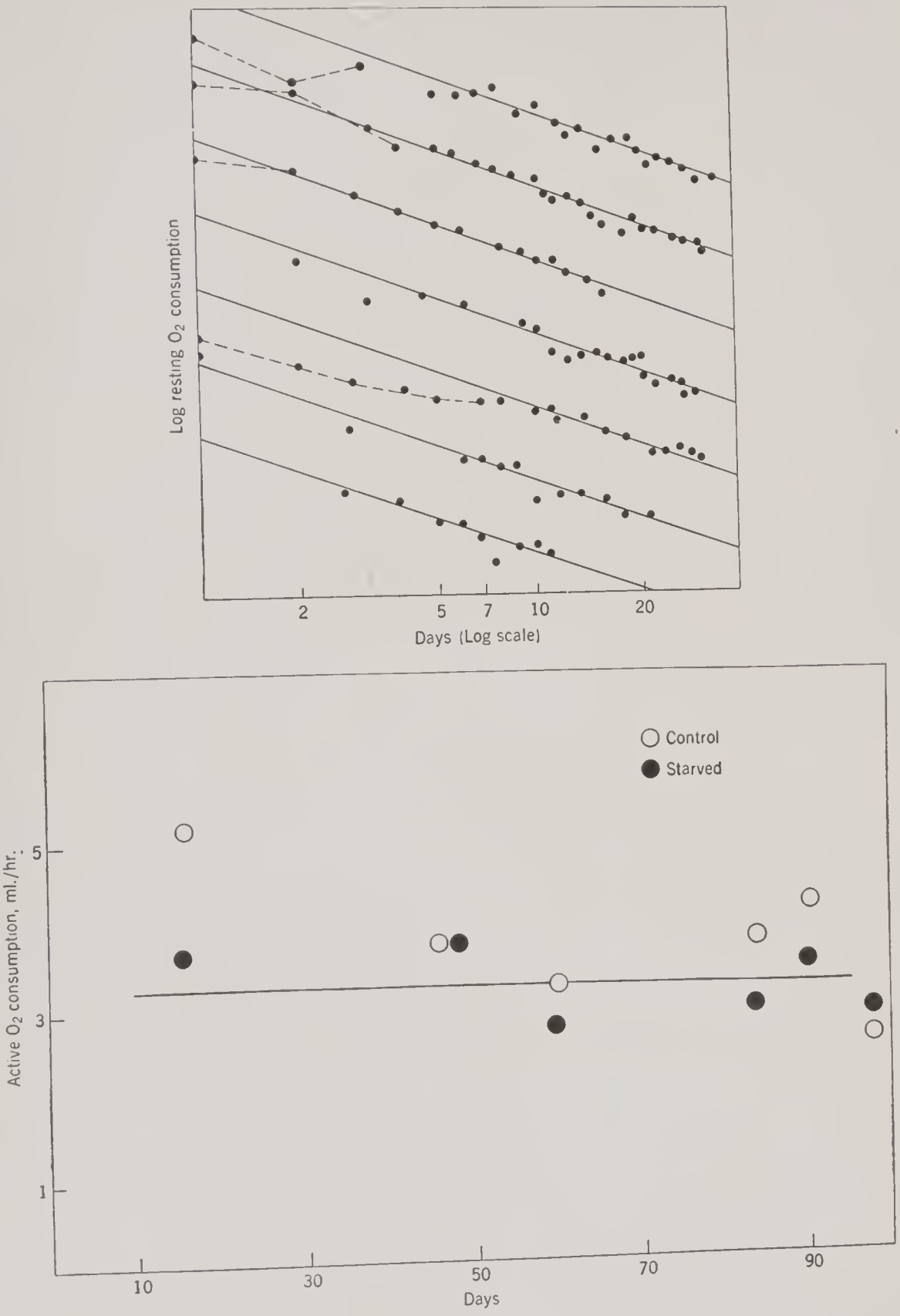


FIG. 16. The effect of starvation on the metabolic rate. Upper, standard metabolism of *Protopterus aethiopicus* (Smith, 1935b); lower, active metabolism of *Salmo gairdnerii* (unpublished data of I. Barrett).

dependence are to be found in the literature because fish are readily provoked to respire at a near maximum rate just by the handling they receive when putting them in a respiration chamber (Black *et al.*, 1939).

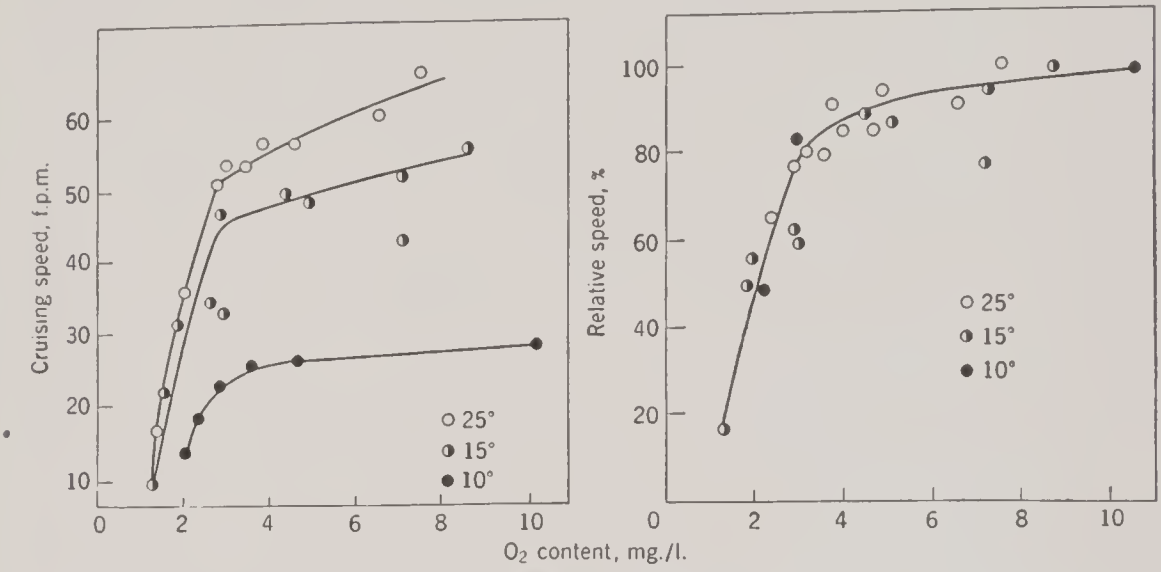


FIG. 17. The cruising speed of *Perca flavescens* in relation to temperature and oxygen content (unpublished data of R. G. Ferguson). The method used to determine cruising speed was that of Fry and Hart (1948b).

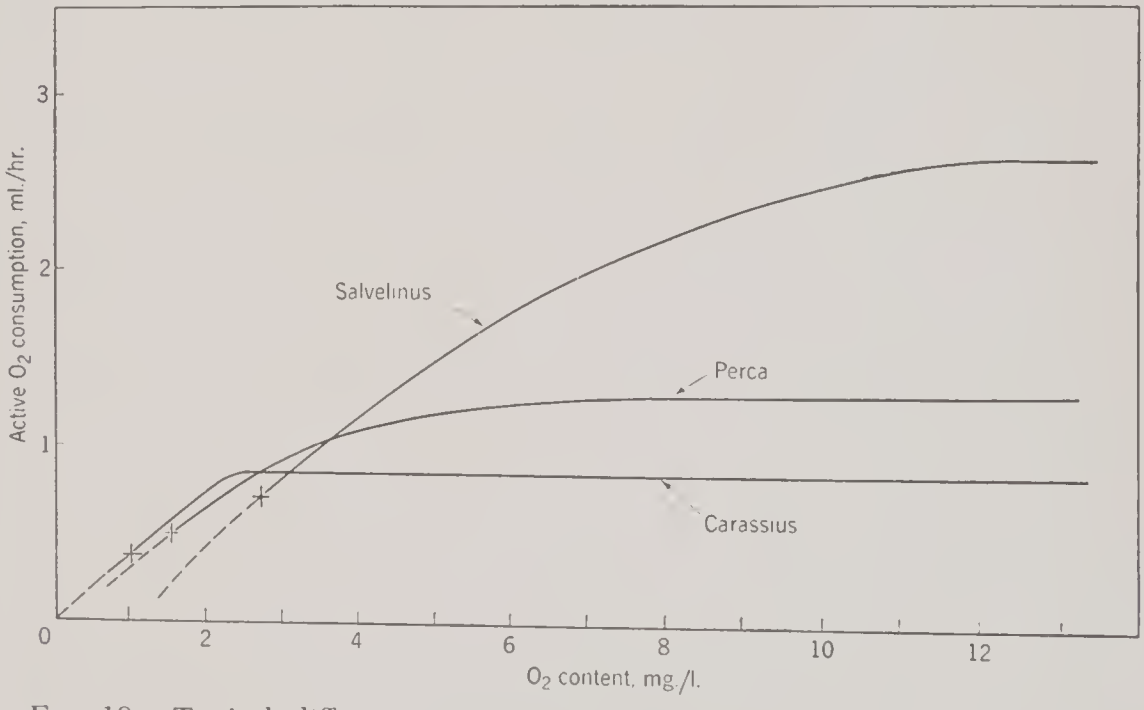


FIG. 18. Typical differences in the respiratory dependence of fish. Curves are for 5 g. fish tested at 20° C. *Salvelinus fontinalis* (Job, 1955); *Carassius auratus* (Fry and Hart, 1948a); *Perca flavescens* (R. G. Ferguson, unpublished).

The general nature of the relation of active respiration to the oxygen content of the medium has already been illustrated by Hart's data presented above (p. 34). The rate of uptake typically decreases curvil-

inearly, and the course of this decrease would often, if extrapolated, bring the rate to zero at a point where there was still a finite amount of oxygen in the water. Such extrapolation is obviously not justified and the true curve must be sigmoid with the flexure occurring at a rate of oxygen uptake below that ordinarily measured (Lindroth, 1940).

Figure 18 illustrates typical differences to be found in the respiratory dependence of fish although it does not quite cover the extremes of this phenomenon shown by Hall's (1929) data. Other curves presented by recent workers are to be found in the following references: Lozinov (1952), Lindroth (1940, 1942), Gibson and Fry (1954). In some species the point of flexure is so low that extrapolation leads directly to the origin, e.g. *Fundulus heteroclitus* (Maloeuf, 1937), *Opsanus tau* (Hall, 1930), the goldfish (Fig. 18). These differences in the nature of the curve are due to differences in the properties of the systems for the presentation, uptake, and transport of oxygen.

An interesting point about Fig. 18 is that respiratory dependence in the species shown there is governed, except for the lowest levels of oxygen, largely by the abilities of the fish to be active rather than by a disability of the system to take up oxygen. Thus, although *Salvelinus fontinalis* still exhibits respiratory dependence at air saturation while *Perca* and *Carassius* are independent at that level, *Salvelinus* is respiring at a much greater rate.

It appears that the major factor in producing the phenomenon of respiratory dependence is the limitation imposed by the maximum amount of water which can be presented to the gills by the ventilatory apparatus. It does not appear to be, as has been occasionally suggested, the affinity of the hemoglobin for oxygen, for the decrease in uptake sets in at a level well above the oxygen pressure that permits the hemoglobin to be saturated. Shepard (1955) has discussed this point in detail for *Salvelinus fontinalis.*

The effect of a reduced oxygen content on the maximum rate of oxygen consumption appears to be independent of size, as Job's data indicate (Fig. 19). However, information is required from more species before it will be safe to generalize.

If the standard (basal) rate of oxygen consumption is considered in relation to the oxygen content there should be an extended range where the respiratory rate is independent of the oxygen concentration. The first change to be expected below this level is an increase in oxygen consumption. This increase in metabolic rate reflects the increase in cost of ventilation (van Dam, 1938) since the response of the fish is to pump more water in order to provide a sufficient oxygen supply to satisfy its resting metabolic rate.

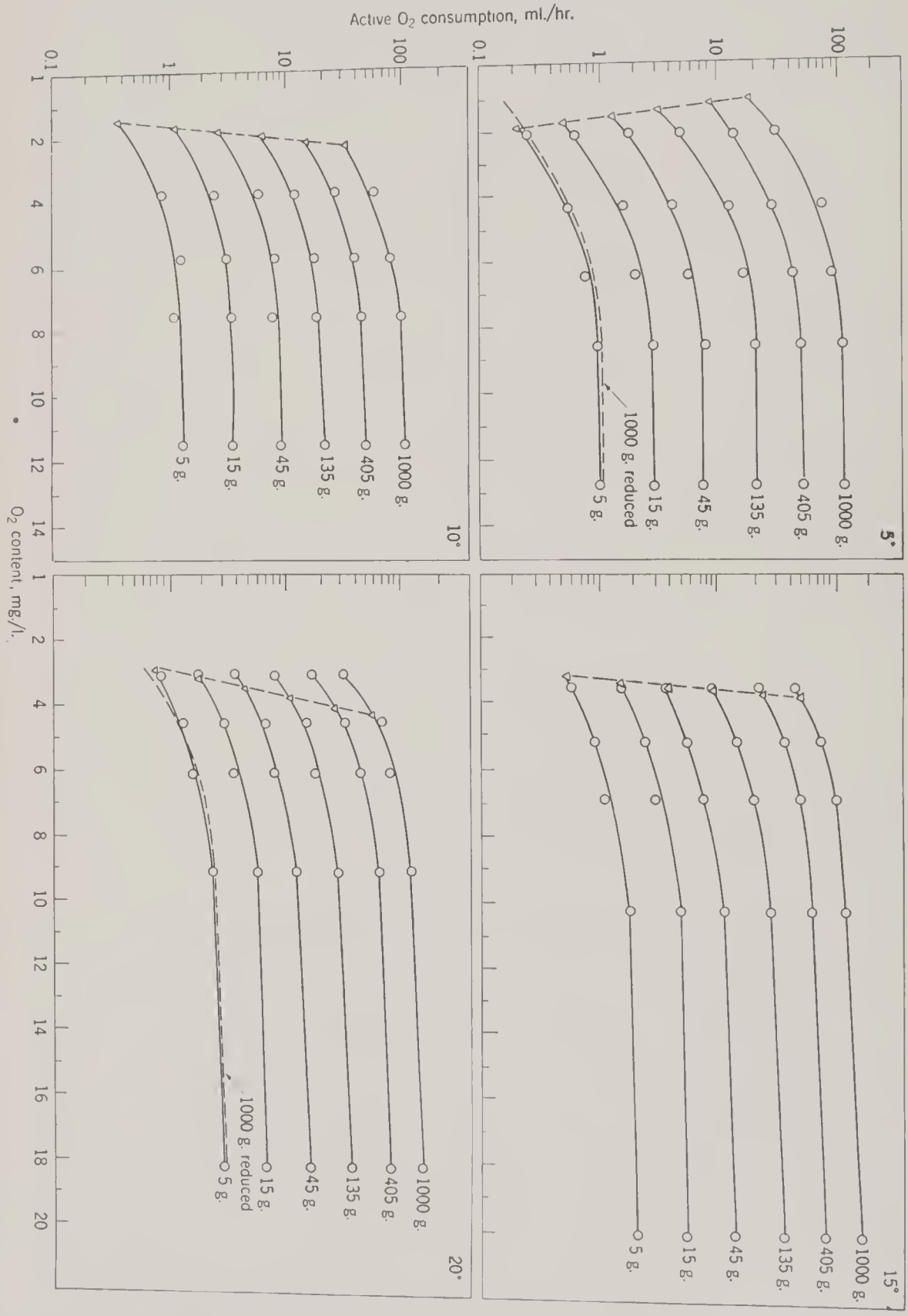


FIG. 19. Respiratory dependence in relation to size. Data for *Salvelinus fontinalis* from Job (1955).

Any reduction of the oxygen content below the level where the active metabolic rate begins to be restricted is probably unfavorable to the species concerned. From the ecological point of view this "incipient limiting level" (the critical level under conditions of activity) can be taken as the point where the oxygen content begins to be unsuitable. The level of the beginning of respiratory dependence as an index of water quality was probably first proposed by Lindroth (1940). It was formalized by Fry (1947) who applied the concept to fish intentionally stimulated to activity. Lozinov (1952) recognized that there is a critical level well above the lethal level but is not at all clear about the effect of activity.

Another important index of the oxygen requirements of a species can be derived from a combination of the active and standard rate of oxygen consumption. This has been termed by Fry (1947) the "level of no excess activity." This index is derived by finding the point on the respiratory dependence curve for active metabolism where the active rate is equal to the standard metabolic rate. These points are indicated by crosses in Fig. 18 for the three species illustrated there. The horizontal bars of the crosses indicate the levels of standard metabolism, the vertical bars the levels of no excess activity which at 20° C. are, respectively, 1.1 mg. O_2/l. for *C. auratus*, 1.7 mg. O_2/l. for *P. flavescens*, and 2.8 mg. O_2/l. for *S. fontinalis*. Of these three species, *C. auratus* has the lowest level of no excess activity not only by virtue of its ability to extract more oxygen from water in which the oxygen content is reduced but also because it has a lower rate of standard metabolism. These points are the levels of no excess activity for individuals acclimated to approximately air saturation levels at the temperature indicated.

Size differences in the tolerance of fish to low oxygen arise from the interplay of the standard metabolic rate and the curve for respiratory dependence. In Fig. 19 the levels of no excess activity for the different sizes of *S. fontinalis* are shown on it by marking the points on the dependence curves where they are depressed to the standard metabolic rate appropriate for the size in question. Since (see p. 36) with increasing temperature there is a slightly greater depression of the slope of the curve relating the metabolic rate to size under conditions of active metabolism in *S. fontinalis*, there is a drift in the effect of size on the level of no excess activity. At 5° C. large trout have a lower level of no excess activity than do smaller ones; at 20° C. the reverse is true. It seems probable therefore that Plehn's (1924) generalization that older fish have a lesser sensitivity to oxygen lack will not always hold.

As pointed out above, the level of no excess activity can be taken as a conservative estimate of the asphyxial level. Conversely, if the level

of oxygen at which a given species is asphyxiated is determined, the rate to which the active oxygen consumption is reduced at the beginning of asphyxia can be taken as an estimate of the basal metabolic rate.

The lethal level of oxygen concentration depends on the previous oxygen regime as is indicated in Fig. 20. Shepard (1955) considers this shift to be largely if not entirely due to the enhancement of the ability to take up oxygen from low concentrations. He found no evidence of any improvement in the ability of *Salvelinus fontinalis* to exist anaerobically as measured by the time that individuals could survive in the absence of oxygen. He did find a change in the ability to take up oxygen which would account approximately for the shift in the lethal

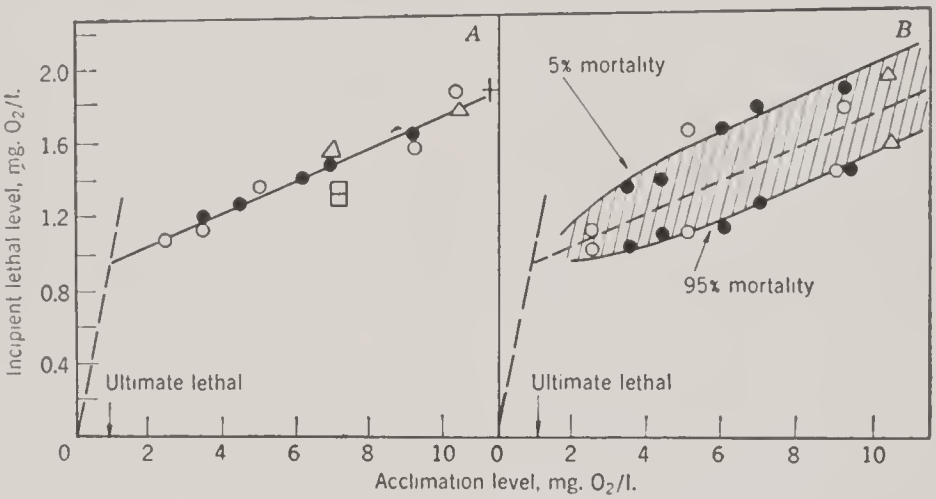


FIG. 20. Lethal level of oxygen in relation to the previous oxygen regime (*Salvelinus fontinalis* at 10° C.) (from Shepard, 1955).

curve. He presumed the process of acclimation was similar to that in mammals where it is largely due to an increase in the oxygen transport capacity of the blood. Acclimation to low oxygen is a fairly rapid process. In *Salvelinus fontinalis*, at 10° C., for a change from 10 mg. O_2/l. to 4 mg. O_2/l., it is half complete in active fish by above 75 hours and is complete by 300 hours.

The limiting effect of the oxygen supply may have special significance in the case of embryos, at least of oviparous species. In the developing egg the metabolism required to organize the yolk into embryo provides a high oxygen demand (see Chap. VIII). To satisfy this demand fully a high ambient level of oxygen is often required. During the early stages, the oxygen requirement of the growing embryo may overpass the extension of the respiratory surface which at this stage is the vascular surface of the yolk sac. Thus even at air saturation there may be a reduction in the rate of oxygen uptake before this stage is completed

and growth may be temporarily slowed down. At later stages the restriction may be brought about by the limiting surface of the egg capsule. Figure 21 from Hayes *et al.* (1951) shows clearly how the capsule may restrict the passage of oxygen. The larvae freed of their egg capsule took up oxygen at the maximum rate displayed by the encapsulated larvae (approximately 35 mm. ^{3}/10 eggs/hour) at a partial pressure which was 50 mm. Hg lower. Lindroth (1942) clearly recognized this restriction and pointed out that the most dangerous time for

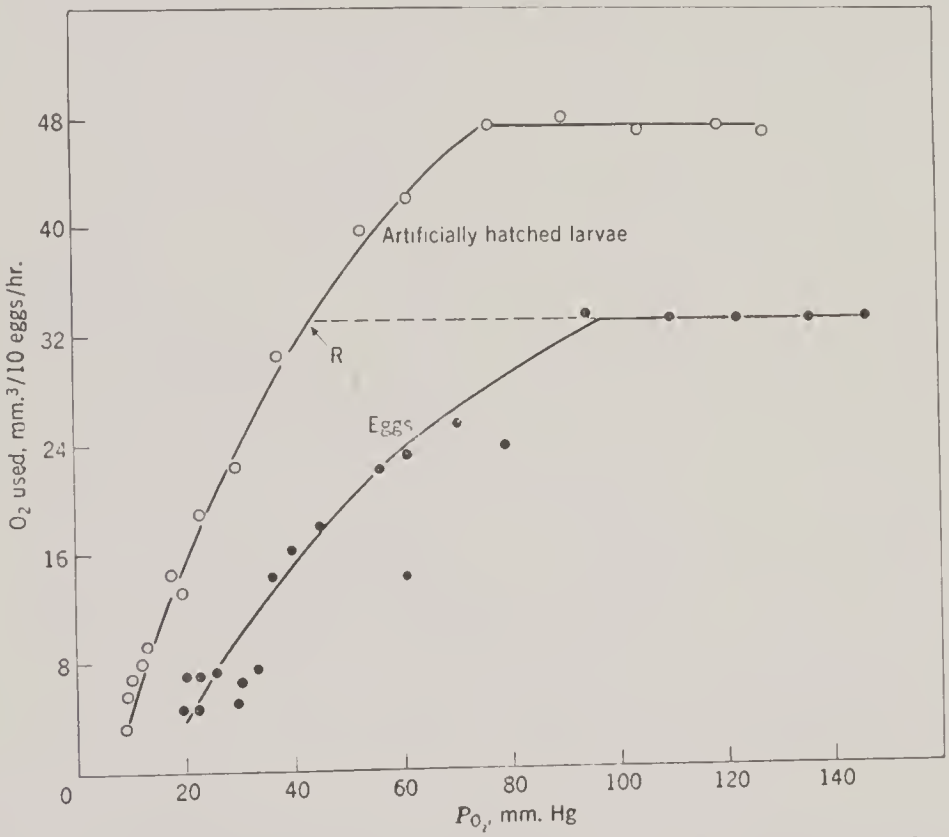


FIG. 21. The limiting effect of the egg capsule (*Salmo salar* at 10° C.) (from Hayes *et al.*, 1951).

developing eggs as far as oxygen lack was concerned comes just prior to hatching.

The energy required for the activity of development, just like the energy required for physical activity, can apparently be limited without damage to the organism. Thus restriction of the oxygen supply for the developing egg, as least when this restriction concerns only the upper region of respiratory dependence, probably does no more than slow down the development of the organism. In such a case oxygen is operating as a limiting factor in the true Blackmannian sense (Blackman, 1905). Apropos of this point Hayes *et al.* (1951) allowed eggs to respire at levels of oxygen which reduced the rate of oxygen uptake to just over one-third of the normal rate for 2 hours. On being returned to water

of normal oxygen content these eggs showed no indication of having accumulated an oxygen debt. On the contrary they continued at the reduced level for the first 15 minutes and then returned to the normal rate. The best interpretation of this observation appears to be that the activity of development had been temporarily reduced. Eggs developing at reduced levels of oxygen have been observed to develop more slowly.

One of the deleterious effects of high temperature on developing eggs may be the inability of the embryo to satsify its augmented oxygen requirements with the supply that can pass through the capsule. This is probably the reason why development may be somewhat slow at higher temperatures. From physical considerations smaller eggs should be less affected by low levels of oxygen whether these are absolute or whether they are relative, brought about by the effect of high temperature on metabolism. Thus Amberson and Armstrong (1933) reported that 5–12-day-old embryos of *Fundulus heteroclitus* which are relatively small can increase their rate of oxygen consumption at 22° C. by at least six-fold if stimulated. The egg capsule clearly does not limit the passage of oxygen to that required for the normal consumption in this case.

E. The Effect of Carbon Dioxide on Oxygen Consumption

This is a question that cannot be dealt with at all satisfactorily from the information at present available. What experimental work has been reported is on the whole fragmentary. Moreover there are two problems. One is the effect of carbon dioxide on the utilization of oxygen from the water respired. The other is the effect of changes in utilization on the rate of oxygen consumption. Thus there will not be any simple response on the part of the organism until acute conditions are reached. The first response will often be an increase in oxygen consumption caused by the increase in ventilation and transport necessary to compensate for the decreased utilization as van Dam (1938) has shown.

The decrease in the utilization of oxygen brought about by an increase in free carbon dioxide has been dealt with by Hall (1931), Meyer (1935), and van Dam (1938). They all agree that increasing partial pressures of carbon dioxide lead to a decrease in the percentage of oxygen utilized. Van Dam has further demonstrated that the decrease in utilization is greater than can be accounted for on the basis of increase in ventilation alone. Specific differences in the effect of carbon dioxide have been found. The rainbow trout responds markedly at a partial pressure of carbon dioxide of approximately 10 mm. Hg whereas the utilization of oxygen by the eel is not affected by the p_{CO_2} up to

approximately 40 mm. Hg according to van Dam (1938) although he found one eel to react to partial pressures of CO_2 of more than 20 mm. Hg by apnea.

There has been little work as yet on the effect of free carbon dioxide on the rate of active oxygen consumption. Basu (personal communication) has obtained preliminary data for *S. fontinalis* at 10° C. which indicate that all pressures of free CO_2 depress the active rate of oxygen consumption in proportion to their level, and that the active rate is depressed to the requirements for standard metabolism as determined by Job (1955) at a p_{CO_2} of about 25 mm. Hg. Black's (1945) data for *Umbra limi* approximate to the active rate of oxygen consumption and in this species there is no marked drop until the partial pressure of carbon dioxide exceeds 100 mm. Hg. Such a slight response to CO_2 is characteristic of many species. Basu (1951) measured the rate of oxygen consumption of three Indian carps, *Catla catla, Labeo rohita,* and *Cirrhina mrigala* together with the common goldfish and found them all to be essentially unaffected by partial pressures of CO_2 up to 100 mm. Hg.

It would appear likely that, except for the most sensitive species, the ordinary environmental levels of carbon dioxide have little influence on the rate of oxygen consumption but considerable work needs to be done on the subject.

Hydrogen ion concentration affects the utilization of oxygen in the same way as does CO_2 (Hall, 1931; Meyer, 1935) but the effects are not completely equivalent. Leiner (1937) observed also a sharp increase in the oxygen consumption of the seahorse, *Hippocampus brevirostris,* when sodium bicarbonate was added to sea water.

F. The Effect of Temperature on the Metabolic Rate

The effect of temperature must be considered particularly for the two extremes of metabolism, the active and the standard. The intermediate case where the animal is exhibiting spontaneous activity is discussed briefly under the heading "routine metabolism."

1. Standard Metabolism

For standard metabolism the conventional relation of an ever-increasing rate with increasing temperature holds as is illustrated in Fig. 22. The rates are shown on a semilogarithmic grid here to stress the relative rate of increase. In conformity with the often observed phenomenon of the diminution of the Q_{10} with increasing temperature the lines on the semilogarithmic plot ordinarily become less and less steep. The line

for *Gobio* is an exeeption in this respeet among the examples shown. Each species appears to have a linc with a eharacteristic slope for a given region of temperature. Thus *Salvelinus fontinalis,* which is a eold stenotherm, shows a relatively high and little changing metabolie rate from 5° C. to 20° C. whereas *Carassius,* which does not approaeh its lethal temperature until 40° C., shows a relatively steep curve in the lowcr range of temperatures and the eurve docs not begin to flatten until the higher temperatures are attained. *Perca,* with an ultimate upper lethal

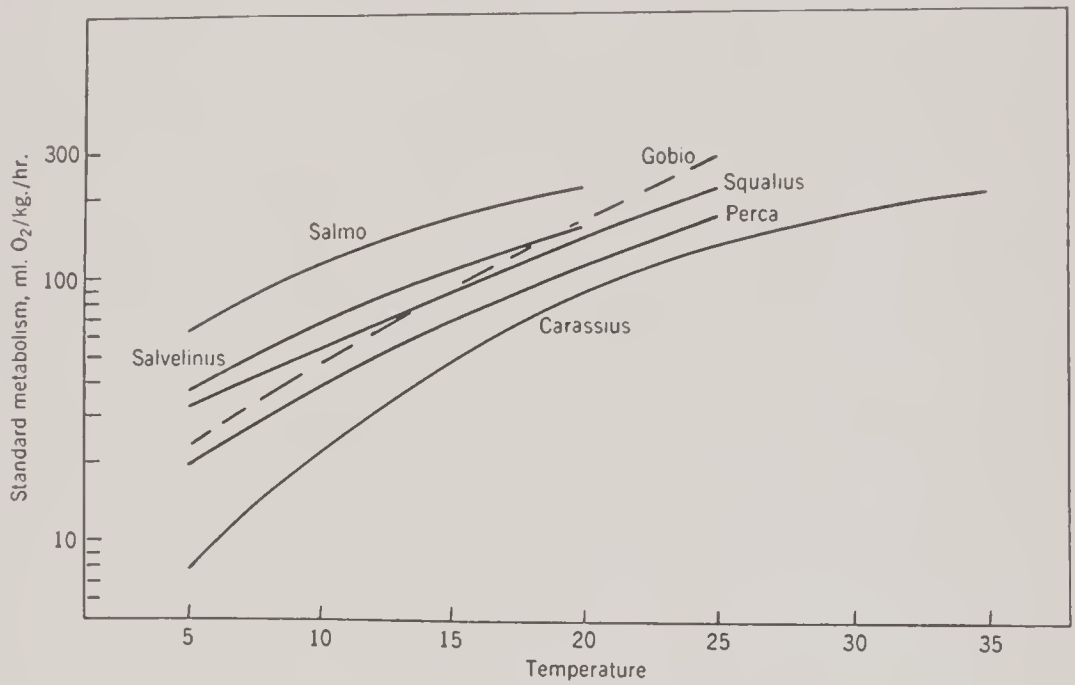


FIG. 22. The relation of standard metabolism to temperature in thermally acclimated fish. *Salmo gairdnerii (Trutta iridea), Squalius cephalus,* and *Gobio fluviatilis* from Flörke *et al.* (1954); *Perca flavescens,* unpublished data of R. G. Ferguson; *Carassius auratus* from Fry and Hart (1948). All curves based on specimens of the order of 5 g.

temperature of approximately 33° C., is intermediate. Lciner (1938) and Fry (1947) show other data from the literature whieh display this same effect. Schlieper (1952) has reeently eommented on this phenomenon and has extended this prineiple to poikilotherms in general, pointing out that the eold stcnotherms have a relatively high metabolie rate at low temperatures; he suggests this is the reason for their low lethal temperatures.

2. Routine Metabolism

The term, standard metabolism, as diseussed abovc has becn eonfined as far as possiblc to the resting rate in thc post-absorptive stage. Ccrtain measures of metabolism havc been taken to reflect minor ehanges in

routine physical activity. This appears to be so in Schmein-Engberding's (1953) recent measurements, for example; he reports for a series of five species of fish a relation between the thermal preferendum and the rate of change of oxygen consumption with increase in temperature. He found that for an interval above the preferred temperature (determined at a low acclimation temperature) there is no increase in the metabolic rate

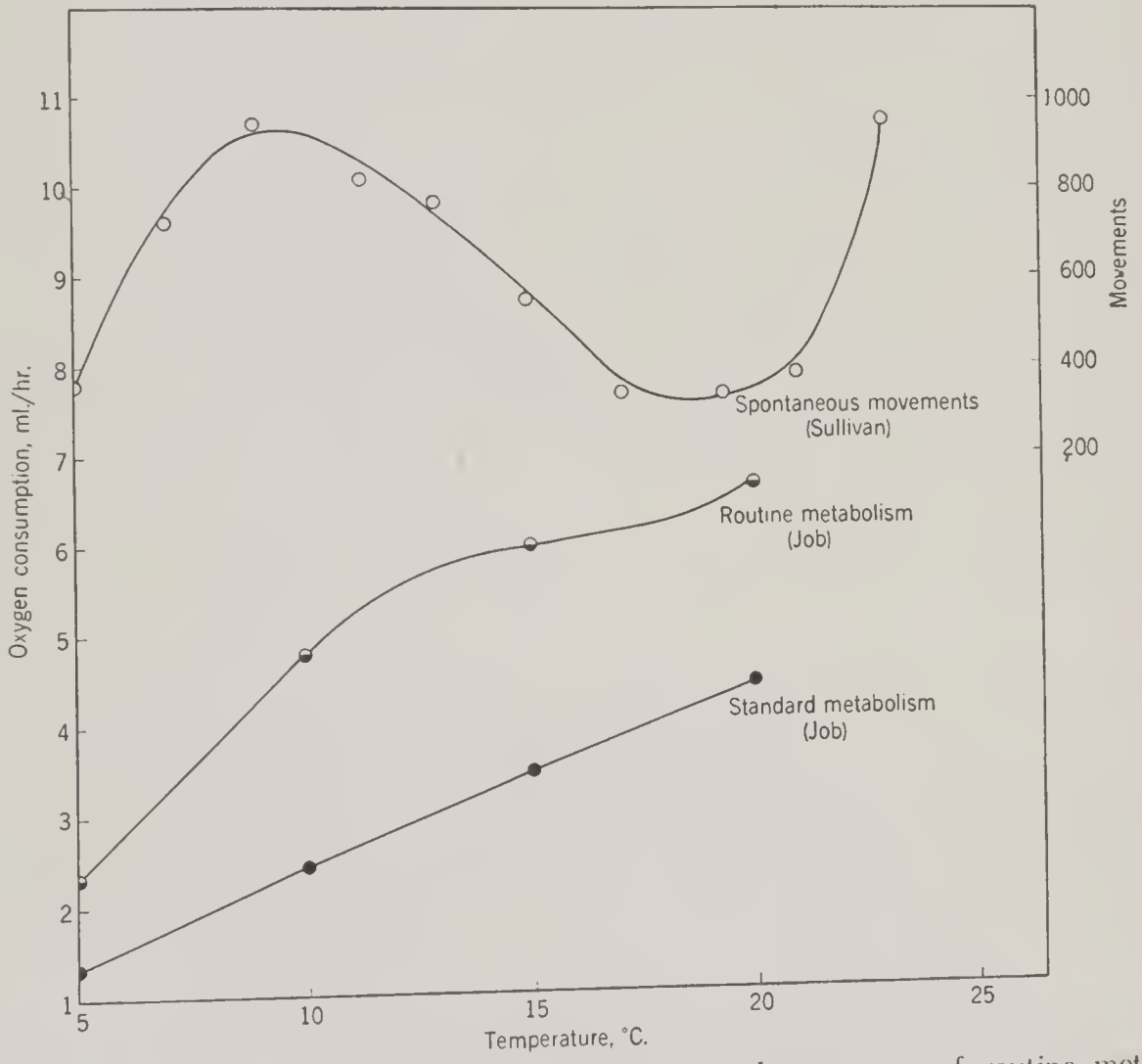


FIG. 23. The effect of spontaneous activity on the response of routine metabolism to temperature in *Salvelinus fontinalis*. Data from Sullivan (1949) and Job (1955). The metabolic data are for 15 g. fish.

with increasing temperature. Subsequently, after an interval of a few degrees, the rate again proceeds to rise. This behavior appears to be a reflection of the spontaneous activity of the fish and was interpreted as such by him since he also made observations on narcotized fish and found no such leveling of the metabolic rate with them. However, he did not publish his absolute values so that other data are offered here to illustrate this phenomenon. Sullivan (1949) reported observations of

herself and K. C. Fisher of the type referred to in Sullivan and Fisher (1947) on spontaneous movements in *Salvelinus fontinalis;* the frequency of such movements in fish carefully sheltered from external stimuli increased with increasing temperature up to the region of the preferred temperature. At higher temperatures the frequency of movements dropped until a point a few degrees short of the lethal temperature was reached. At this point the frequency of spontaneous movements increased again, this second increase continuing up to the lethal temperature. Job's (1955) data for 15 g. fish both for the routine metabolism (the mean metabolic rate over a 24-hour cycle in a darkened respiration chamber) and for standard metabolism (the lowest point in the 24-hour cycle) are presented in Fig. 23 together with Sullivan's (1949) data for spontaneous activity taken from her Fig. 9a. The rise with increasing temperature in Job's data for routine metabolism begins to slacken off between 10 and 15° C. which is in the region of the lower zone of the preferred temperature of this species (Sullivan and Fisher, 1953) and also not far from the temperature where Sullivan reported that the decrease in spontaneous activity begins. Since Job did not carry his observations above 20° C. the second rise has not been explored. However the line for routine respiration must be flexed upwards again between 15 and 20° C. to pass through the 20° value. In contrast to the sigmoid nature of the curve for routine metabolism which embraces spontaneous activity, the relation of standard metabolism to temperature in 15 g. specimens of *S. fontinalis* is almost exactly linear over the range 5–20° C. as the lower line in Fig. 23 indicates.

The effect of temperature on spontaneous activity described above is characteristic of a stable condition in which the fish has become adjusted to the temperature in question (Sullivan, 1954). The short term effect of a sudden change in temperature has been dealt with earlier (p. 33).

3. Active Metabolism

The active metabolic rate in relation to temperature does not necessarily follow a course parallel to the curve for the standard rate. Figure 24 shows, respectively, for two cold stenothermal and two eurythermal species of fish the range of diversity to be found in the course of the active and standard metabolic rates in relation to temperature. The active rate may go on increasing until the fish reaches its upper lethal limit of temperature as in *Salmo* and *Ameiurus* or it may reach a plateau as it appears to do in *Carassius* or, as in *S. namaycush,* it may be actually depressed at the higher temperatures within the biokinetic range, at least when the dissolved oxygen does not exceed air saturation. In contrast

the standard metabolic rates of the 4 species illustrated in Fig. 24 show a qualitative uniformity of response. The consequence is that the difference between the levels of active and standard metabolism in relation to temperature shows a diversity of pattern as great as that shown by

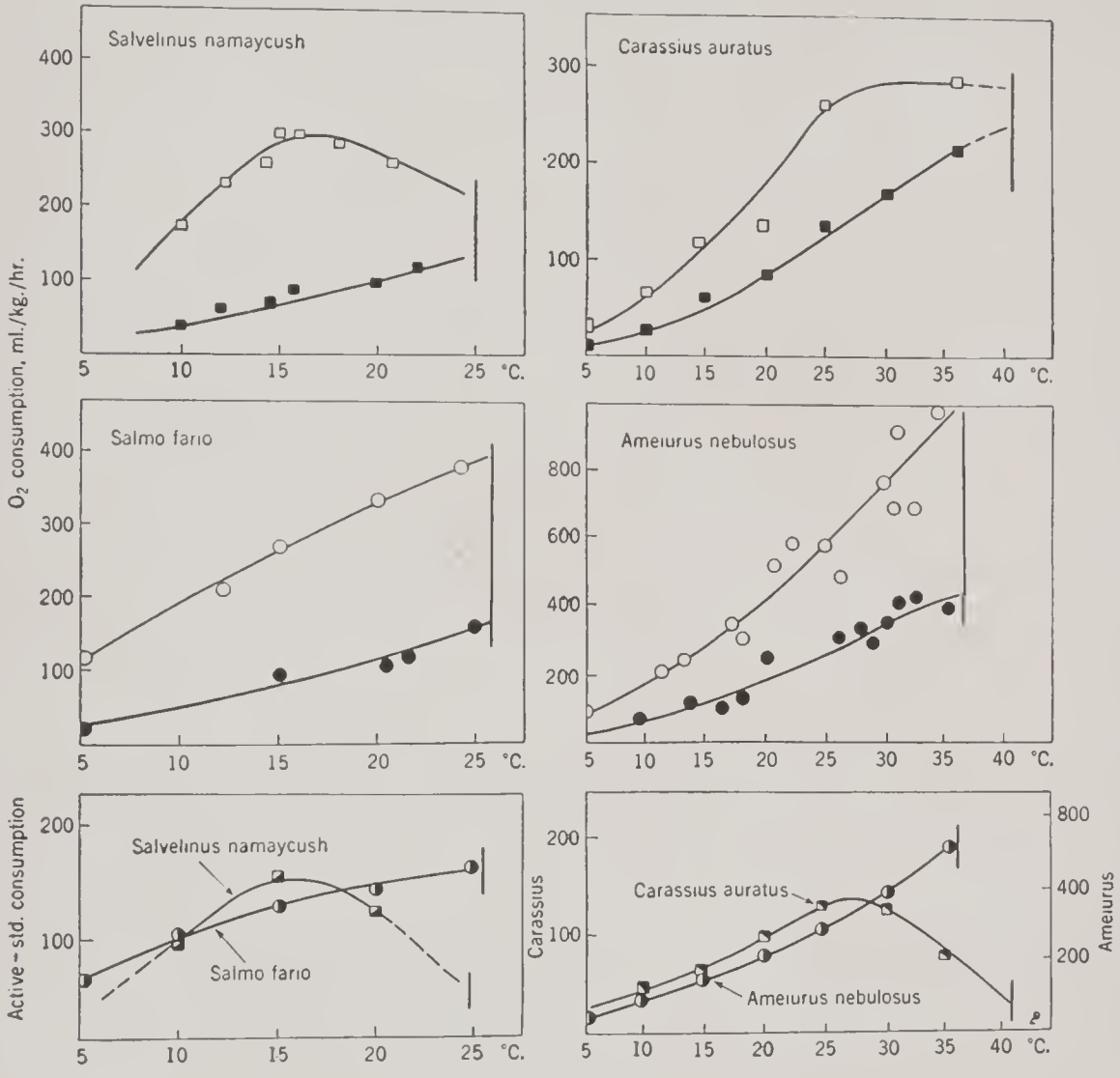


FIG. 24. Active and standard rates of metabolism in thermally acclimated fish. Note that the scales differ for different species. Data from the following sources: *Salvelinus namaycush* (Gibson and Fry, 1954); *Salmo fario* (unpublished data of J. M. Graham); *Carassius auratus* (Fry and Hart, 1948a); *Ameiurus nebulosus* (data of Paul and Fry in Fry, 1947).

the active metabolic rate. These diverse trends of the difference between the active and the standard metabolic rates are shown in the lower panels of Fig. 24. In two of the species (*S. namaycush* and *C. auratus*) the difference between the active and the standard rates is greatest at some intermediate temperature and decreases at higher temperatures. In the other two (*S. fario* and *A. nebulosus*) the difference increases right up to the lethal temperature.

The significance of the difference between the active and the standard rates of metabolism is that it appears to express well the respiration available for activity, and it has been termed by Fry (1947) "the scope for activity." The square root of the scope for activity correlates well with the maximum steady rate of swimming of fish as Fig. 25 shows.

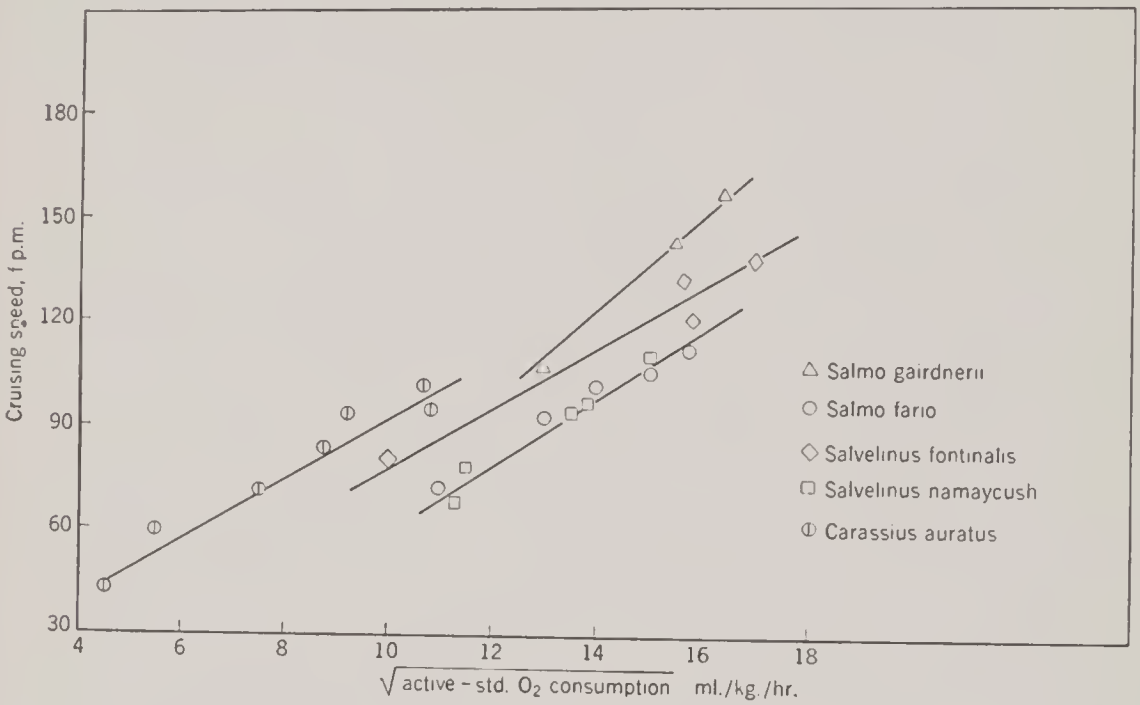


FIG. 25. Relation between swimming rate and scope for activity in various species. *Salmo gairdnerii, Salmo fario,* and *Salvelinus fontinalis* (unpublished data of J. M. Graham); *Salvelinus namaycush* (Gibson and Fry, 1954); *Carassius auratus* (Fry, 1947).

The square root is that transformation of the scope which would be expected to show a linear correlation if the scope represented the power available to overcome the internal and external viscosities which oppose movement. Since the greatest scope for activity does not necessarily come at the highest temperature at which a given species can exist, the optimum temperature for activity of a species does not necessarily bear any relation to its lethal temperature. Thus *Salmo fario* would be expected to be more active in warmer water than *Salvelinus namaycush,* an expectation which is consistent with the behavior of these species in nature. The same is true of a comparison between *Ameiurus* and *Carassius.* In spite of its lower lethal temperature *Ameiurus* is more nearly a tropical species than is *Carassius.*

It would appear likely that death at high temperature is not necessarily the same type of temperature death in all species. Gibson (1954) has shown for *Lebistes reticulatus* that there are probably three different causes for death at high temperatures each cause being operative over

a particular lethal range. Loeb and Wasteneys (1911) observed that the removal of osmotic stress increased the thermal resistance in *Fundulus heteroclitus*. Halsband (1953) was able to raise the lethal temperature of *Salmo gairdnerii* at least two centigrade degrees by increasing the calcium and magnesium content of the water. Some species, such as *S. fario*, ordinarily die at relatively low temperatures but have respir-

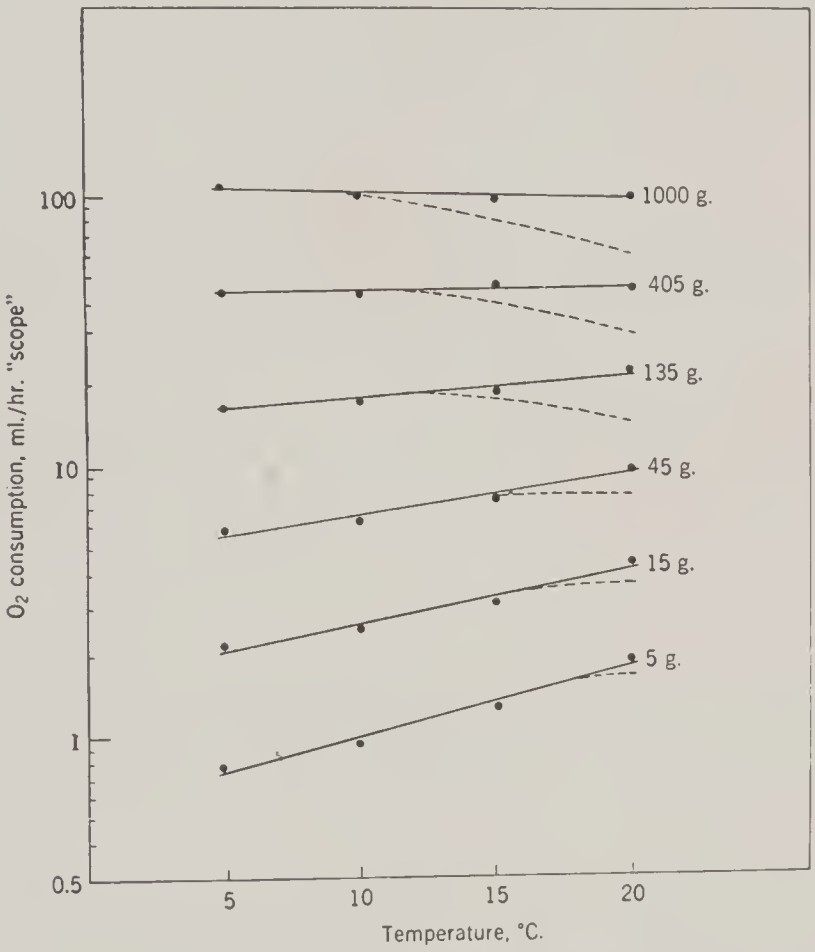


FIG. 26. Scope for activity of *Salvelinus fontinalis* in relation to size and temperature in the presence of high oxygen. The interrupted lines indicate the scope when the partial pressure of oxygen is limited to air saturation (from Job, 1955).

atory systems that allow a still high active transport of oxygen in comparison with the standard rate at that temperature. In a less stringent environment, these might display a relation between the standard and active rates of oxygen uptake such as is shown by *C. auratus*.

The scope for activity may be more or less stable over a wide range of temperature since on the one hand the basal requirements of the organism are less at a lower temperature where the active rate may be somewhat lower. On the other hand, at higher temperatures, the basal requirements are increased as well as the active rate. Thus a poikilotherm often has a wider range of thermal indifference with respect to activity

than might be expected. Job's data for *S. fontinalis* (Fig. 26) illustrate this phenomenon well besides showing, as was intended, the effect of size on the relation of the scope for activity to temperature. Job found a complete reversal in the relation of the scope for activity to temperature in *S. fontinalis* over the size range of 5–1000 g. The smallest fish showed an increasing scope from 5° C. up to 20° C., the largest fish showed a decreasing scope. This change was brought about by a relatively greater reduction in the active rate in larger fish as the temperature increased.

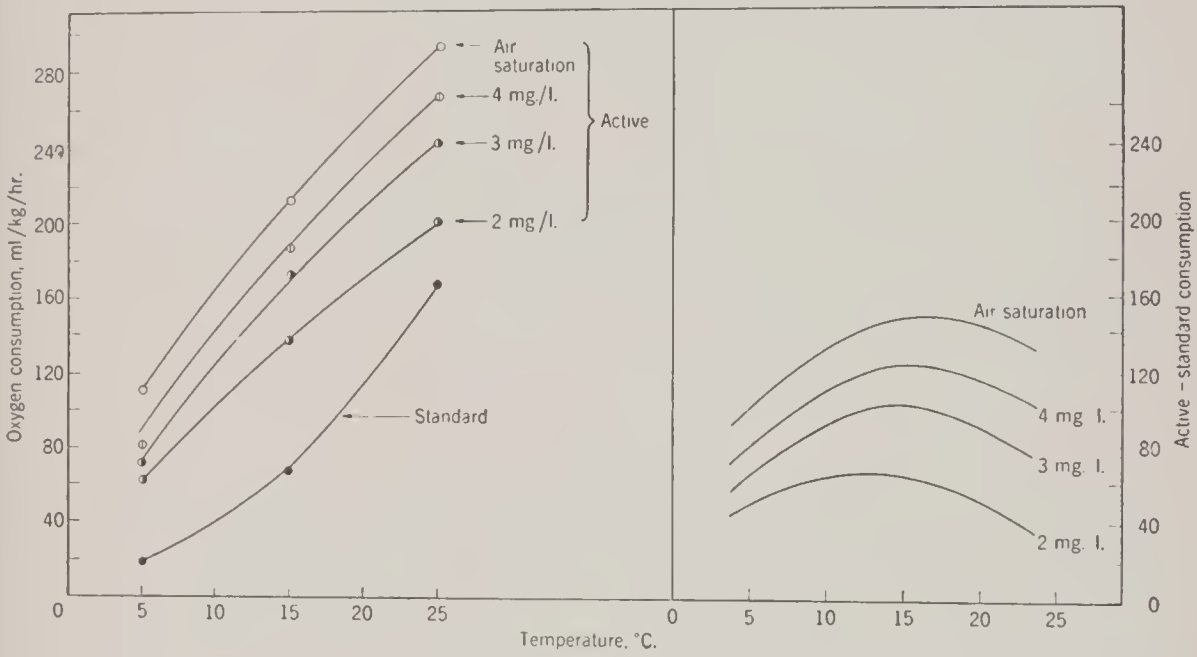


FIG. 27. The combined effect of oxygen content and temperature on oxygen consumption in *Perca flavescens* (unpublished data of R. G. Ferguson).

G. The Interaction between Temperature and Oxygen Content

The combined effects of oxygen content and temperature with respect to the level of no excess activity have been pointed out above (p. 42) in connection with Fig. 19. The interaction between oxygen content and temperature on active metabolism is illustrated in Fig. 27. Similar data are available for the goldfish (Fry and Hart, 1948a), *Salvelinus fontinalis* (Graham, 1949; Job, 1955), and *S. namaycush* (Gibson and Fry, 1954). The left panel of the figure shows the standard rate of oxygen consumption and the maximum rate both at air saturation and when limited by various levels of oxygen content. The right panel takes the standard metabolic rate at each temperature as the base line and shows the distance to which the active rate extends above this at each temperature and level of oxygen content. Thus in this panel, the scope for

activity may be read off directly as in the lowermost panels of Fig. 24 and in Fig. 26.

Figure 27 shows that in *P. flavescens* reduction of the oxygen content of the water to 4 mg./l. reduces the active metabolic rate and thus the scope for activity at all temperatures investigated. Thus the metabolic data correlate well with the cruising speed data for the same species given in Fig. 17. Further reductions in the oxygen content below 4 mg./l. of course produce further reductions in the active metabolic rate until (as may be estimated from the trend of the lines in Fig. 27) a reduction of the oxygen content to 1 mg./l. would, at least at the upper temperatures, depress the active rate to a level that would not meet the standard requirements. Such a reduction would lead to a lethal condition as has been mentioned earlier (p. 42). Just as the level of no excess activity rises with increasing temperature so also the scope for activity tends to be more severely restricted at higher temperatures as the right hand panel of the figure indicates. Thus any optimum, conditioned by a reduced oxygen content, for the scope for activity becomes displaced towards a lower temperature.

H. Summary

The measurement of the metabolic rate of fishes requires that careful attention be given to the past as well as the present state of activity of the subject under investigation. Fish are easily provoked to consume oxygen at a near maximum rate and are slow to reduce their metabolic rate after activity or excitement. The resting metabolic rate is also influenced greatly by feeding or starvation.

Most species of fish show a standard rate of oxygen consumption that is related to the weight raised to a power of approximately 0.8. Certain species, however, have been found to conform to the surface area law. The relation of the standard rate of oxygen consumption to size is essentially independent of temperature. The relation of the active metabolic rate to size is of the same order as the standard rate in the one species where it has been investigated but was found to change somewhat with temperature, larger animals not quite keeping pace with smaller ones.

The resting metabolism of fishes is adequately handled by their respiratory system often down to a concentration of ambient oxygen of the order of 1 mg./l. The lowest level of oxygen at which the resting metabolism can be met in a given species depends as much on the oxygen demand of the species as on the characteristics of its respiratory system. The active metabolism of fish is much more strongly dependent

on the oxygen content of the water. The degree of dependence of the active rate is related to the oxygen demand as much as to the specific character of the respiratory system. The data for *Opsanus tau,* however (Hall, 1929), show an exception to this rule.

The activity of which the fish is capable is correlated with the difference between the standard and active rates of metabolism. Thus any partial pressure of oxygen which reduces the active rate of metabolism reduces the activity of the fish and places the species in question at a disadvantage. At a particular low partial pressure of oxygen the active metabolic rate of the fish is reduced down to the bare maintenance requirements and the organism is no longer able to perform external work. Below this level a further reduction in the partial pressure of oxygen quickly leads to death.

The active rate of oxygen consumption is probably also affected by other natural fluctuations of the quality of water such as the carbon dioxide content and acidity but information on this subject is as yet fragmentary. The standard rate has been shown to increase in certain species in response to an increase in the external concentration of carbon dioxide. The increase has been attributed to the increased cost of respiration.

The rate of standard metabolism increases continuously with increasing temperature up to the lethal temperature in animals allowed sufficient time for adjustment and not subject to other environmental stress. The active rate of metabolism may, however, level off or even decline at a temperature well below the upper lethal limit. The scope for activity, which depends on the differences between the active and standard metabolic rates, varies with temperature in a way that cannot be predicted from the standard metabolic rate. It may be somewhat uniform over a more or less extended range of temperature or it may show a well-defined optimum at a temperature not necessarily related to the lethal temperature.

The effect of a given reduction in the oxygen content of the water will differ at different temperatures. The general effect will be that a given reduction will restrict activity most severely at the highest temperature and that the optimum temperature when conditioned by a reduced oxygen content will be lower than the unconditioned optimum.

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Chapter I: Part 2

AIR BREATHING

G. S. Carter

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I. INTRODUCTION

It is only in exceptional circumstances, where the environment or the fish's habits make aquatic respiration impossible or inefficient, that we find fish that have departed from the normal aquatic respiration through the gills and have evolved organs adapted for breathing atmospheric oxygen. The exceptional environmental circumstances in which this occurs are of two kinds. Either the water in which the fish lives is liable to deoxygenation and it can then obtain the oxygen it requires only by breaking the surface and breathing air; or the water is liable to dry up in periods of drought and the fish is forced at such times to live out of the water. There are also some fish, such as the eel (*Anguilla*), the climbing perch (*Anabas*), and the mudskipper (*Periophthalmus*), that have habits which lead them to migrate from the water at times when they are not in danger from drought.

The fishes in which organs for air breathing have been evolved fall, from the point of view of systematics, into two groups. Paired lungs were evolved in the early bony fishes, the Osteichthyes (or perhaps in their ancestors, the Placodermi) and these lungs are still used as air-

breathing organs in some surviving primitive members of the group—Dipnoi, *Polypterus*. Since the amphibians possess lungs, they must also presumably have been present in the early Crossopterygii, from which the amphibians are descended, but there is no functional lung in the modern crosspterygian, *Latimeria*. In all actinopterygians except *Polypterus* the lungs have, if we may accept the homology, been replaced by the air bladder. This is respiratory in the Holostei (*Amia* and *Lepidosteus*), but in the higher actinopterygians (Teleostei) it typically has the very different function of hydrostatic control (see Vol. II, Chapter IV).

A wide variety of teleostean fishes, however, which need to breathe air owing to the special conditions of their life, have evolved new organs. These form the second of our two groups of air-breathing fishes. In some the air bladder has been secondarily adapted as an air-breathing organ; in others new organs of aerial respiration have been evolved in various parts of the body; in a few the gills are themselves used for breathing air; and in still others that leave the water, the skin is respiratory.

The interpolation of a new respiratory organ in the body at some place distant from the gills has led to modification of the circulation in many of these fishes. One example of this occurred in the osteichthyan ancestry of the terrestrial vertebrates and gave rise to their figure 8-shaped circulation (see p. 95). For this reason the study of air breathing in the fishes is of special interest because of the light it throws on the course of evolution at the time when the vertebrates left the water to become terrestrial.

II. OCCURRENCE

A. Deoxygenation

In the surface waters of the sea, wind disturbance is sufficient to maintain the oxygenation of the water. Deeper waters may in certain regions become deoxygenated (e.g. the middle waters of parts of the Atlantic or the bottom waters of the Black Sea) but fish in these waters have no opportunity of reaching the surface. Air breathing is therefore rare in marine fish. It has been observed in two dogfishes (*Chiloscyllium indicum* and *griseum*, George 1953) and in a sole of the genus *Achirus* (Breder, 1941). It occurs in young tarpon, *Megalops* (Schlaifer and Breder, 1941), which live in fresh and brackish water pools as well as in the sea, and in one form from the tidal zone mentioned below (p. 67).

In all the tropical regions of the world, deoxygenation is frequent in shallow and stagnant fresh waters, and it is in waters of this type that the great majority of air-breathing fishes are found. Wherever such

waters are protected from wind disturbance by a dense cover of aerial vegetation, and heavily shaded by the same cover so that photosynthesis in them is slight, and where cooling at night is not enough to produce overturn, deoxygenation of the water is bound to occur. Probably, it is also encouraged by rapid absorption of oxygen in the active bacterial decay in the mud below the water. Deoxygenation is in fact typical of such waters wherever the climate is tropical.

Since the only source of oxygen in these waters is diffusion from the air, deoxygenation is always greater in the lower layers of the water, but often layers within an inch or so of the surface may be far from saturated with oxygen or even almost completely deoxygenated. In such conditions small fish may be able to use the aerated surface film by coming to the surface and nibbling at it without taking air. This they may often be seen to do, but for larger fish air breathing is the only means of satisfying their respiratory needs.

In one other environment the occurrence of air breathing has been ascribed to deoxygenation. This is the environment of rapid hill streams in India. In the rainy season these streams are torrential, and their water therefore fully oxygenated, but in dry weather they shrink to series of muddy pools. The evolution of air-breathing organs in some fishes of these streams (*Amblyceps* and some others) has been thought to be in adaptation to the conditions of these dry-weather pools (Hora, 1933).

B. Life Out of the Water

A few fishes habitually leave the water and spend part of their time in the air. The eel (*Anguilla anguilla*) is well known to do so, being sometimes found moving across damp grass while migrating from one body of water to another. Some fishes make trips out of the water for other reasons. The mud skipper (*Periophthalmus*) is commonly to be found on the surface of mud on tropical shores in various parts of the world; and the climbing perch (*Anabas*) climbs above the surface on the sloping trunks of trees and other vegetation. Several of the air-breathing fishes of pools and swamps in southern Asia (*Pseudapocryptes, Saccobranchus,* etc.) are often found out of the water (Das, 1927). Probably the reason for these trips into the air is mainly search for food. It has also been recorded (Hora, 1932) that *Andamia heteroptera,* a fish of the tidal zone of tropical shores in southern Asia, lives in the spray, following the tide and always out of the water.

Lastly, some fishes estivate by burrowing in the mud when the water of the pool or swamp in which they live dries. The dipnoans *Lepidosiren* and *Protopterus, Synbranchus* [1] *marmoratus* (Carter and Beadle,

[1] Better known as *Symbranchus.*

1931), and some Asiatic air-breathing fishes (e.g. *Pseudapocryptes lanceolatus*, Das, 1933, 1934) do so. *Amia calva* has also been found estivating in dried mud (Neill, 1950). When so estivating, the respiration of these fishes must be entirely aerial.

III. AIR-BREATHING ORGANS

A. Proof of Air-Breathing Function

If the fish lives in deoxygenated water, it is clear that any organ of aerial respiration must be internal to the body; use of the external surfaces is only possible where the function of air breathing is to allow the fish to leave the water. In fact only the eel (*Anguilla*), *Periophthalmus*, and its close relative, *Boleophthalmus*, have been shown to carry out a significant part of their respiration by an external organ, the skin. But not every organ within the body that contains gas is necessarily an organ of aerial respiration; the example of the air bladder is enough to make this obvious. To establish the respiratory function of an organ requires considerable evidence. Ideally, these facts should be established:

(1) The epithelia of the organ should have a rich blood supply and the blood should be shown to be better oxygenated after traversing the capillaries of the organ.

(2) There should be a regular exchange of air between the cavity of the organ and the exterior.

(3) The contained air and that excreted from the organ should contain less oxygen and more carbon dioxide than atmospheric air.

Evidence of all these kinds has been obtained for relatively few air-breathing fishes. For many, belief in the reality and significance of their air breathing is based on biological rather than physiological evidence. Such evidence is never valueless and may be convincing. If, for instance, it can be shown that the fish can live in foul water when it is allowed to come to the surface and take air, but dies when prevented from doing so, or if the fish habitually leaves the water to migrate over land, there can be no doubt that it is able to respire aerially. Nevertheless, the evidence must be considered critically, for it is certainly true that many fishes have been said to breathe air on insufficient evidence. Here, in particular, it should be noted that evidence that a fish takes bubbles of air at the surface is not in itself proof of true air breathing. Many fishes, not specially adapted to air breathing, may do this on occasion, taking the air into their mouths and, presumably, using it to improve the aeration of the water of their gill current. These fishes are not considered further here.

Further, the fact that a fish has aerial respiration does not necessarily

mean that it is incapable of aquatic respiration. Many fish that breathe air on occasion do not do so when in water which is fully oxygenated (*Erythrinus,* Carter and Beadle, 1931; *Lepidosteus,* Potter, 1927; *Umbra,* Geyer and Mann, 1939; etc.). On the other hand, some of the fishes that breathe air cannot live by aquatic respiration even in well-oxygenated water (*Lepidosiren,* Fullarton, 1931; *Hoplosternum,* Carter and Beadle, 1931; *Electrophorus,* Carter, 1935; *Saccobranchus,* Das, 1927; etc.). In these the gills are often degenerate. In general, the occurrence of air-breathing organs is accompanied by reduction of the gills. It has been shown (George and Dubale, 1941), by comparison of many air-breathing fishes with water-breathing fishes of the same size, that the respiratory area of the gills in air-breathing forms is on the average less than one-half of that of the others (air-breathing fish, 24–40 mm.^{2} per cubic centimeter volume, others 65–76 mm.^{2}).

B. Position in the Body

Use of the gills for breathing air is rare among air-breathing fishes. This is not surprising. In water the secondary folds on the filaments of the gills are held apart by the current of water passing between them, whereas in the lighter medium of the air they are liable to collapse against each other. The gills, in fact, are ill-adapted to aerial respiration. They could only be effective in air breathing if the secondary folds were stiff enough to stand apart in air. No evidence that this is so in any fish has been given, but a few fish (*Hypopomus* and probably *Synbranchus,* Carter and Beadle, 1931) appear to use their gills for air breathing. They take air into the gill chamber and no accessory respiratory organ has been found in their bodies. They can live in foul water if allowed to reach the surface, and *Synbranchus* certainly breathes air while estivating in its dry-weather burrow.

The accessory air-breathing organs of teleosts are modifications of the epithelia of the alimentary canal or gill chamber, or diverticula from them. The air bladder is used in some Physostomi (*Erythrinus, Umbra, Gymnarchus,* etc.), and the air-breathing organ may occur in almost any part of the alimentary canal—mouth, pharynx, stomach, intestine—or in the gill chamber. Very often the area of epithelium is increased by formation of branched or arboriform outgrowths. The locations of some of the best known of these organs is given in Table I.

C. Exchange of Air

In all the fish that have internal air-breathing organs the air is taken in at the mouth. The fish comes to the surface of the water, breaks it, and takes a bubble of air which is then passed to the organ. Where the

TABLE I

AIR-BREATHING ORGANS IN THE FISHES

Air-Breathing Organ	Circulation		Habitat
	Afferent	Efferent	
I. PHARYNGEAL EPITHELIA OR DIVERTICULA			
Ophicephalus	Aortic arches	Jugular vein	Tropical pools, Asia, Africa
Periophthalmus	Carotid artery	Jugular vein	Muddy tropical shores, Africa, Asia, Australia
Electrophorus (Gymnotus)	Aortic arches	Jugular and anterior cardinal vein	Pools in South America
Monopterus	Aortic arches	Jugular vein	Fresh waters, southern Asia
II. EPITHELIA OR DIVERTICULA OF THE BRANCHIAL CHAMBER			
Clarias	Afferent arches	Efferent arches	Tropical pools, Asia, Africa
Saccobranchus	Afferent arches	Efferent arches	Tropical pools, Asia
Anabas	Efferent arches	Jugular vein	Tropical pools, Asia, Africa
Macropodus	Efferent arches	Jugular vein	Tropical pools, Asia
Osphronemus	Efferent arches	Jugular vein	Fresh waters, southern Asia
Betta	Efferent arches	Jugular vein	Fresh waters, southern Asia
Amphipnous	Aortic arches	Jugular vein	Rivers, etc., southern Asia
III. BRANCHIAL CHAMBER FILLED WITH AIR			
Hypopomus	Afferent arches	Efferent arches	Swamps of South America
Synbranchus	Afferent arches	Efferent arches	Swamps of South America
IV. AIR BLADDER AND LUNG			
Polypterus	Efferent arch, VI	Hepatic vein	Fresh waters, Africa
Amia	Aortic arch, VI	Left ductus Cuvieri	Rivers, North America
Lepidosteus [1]	Coeliac artery	Right posterior cardinal vein	Rivers, North America
Gymnarchus	Efferent arches, V and VI	Direct vein to atrium	Rivers and swamps, Africa
Erythrinus	Coeliac artery	Right posterior cardinal vein	Swamps, South America
Umbra	Coeliac artery	Right posterior cardinal vein	Stagnant waters, Europe, North America
Lepidosiren	Aortic arch, VI	Pulmonary vein	Swamps, South America
Protopterus	Efferent arch, VI	Pulmonary vein	Lakes and swamps, Africa
Neoceratodus	Efferent arch, VI	Pulmonary vein	Rivers, Australia

[1] = *Lepisosteus*

TABLE I *(Continued)*

Air-Breathing Organ	Circulation		Habitat
	Afferent	Efferent	
V. Stomach and Intestine			
Misgurnus	Dorsal aorta	Portal vein	Rivers and pools, Europe and Asia
Doras	—	—	Rivers and swamps, South America
Callichthys	Dorsal aorta	Inter-renal vein	Swamps, South America
Hoplosternum	Dorsal aorta	Inter-renal vein	Swamps, South America
Hypostomus	—	—	Swamps, South America
Ancistrus	Coeliac artery	Inter-renal vein	Swamps, South America
Plecostomus	Coeliac artery	Portal vein	Swamps, South America
VI. Skin			
Anguilla	Normal arteries	Normal veins	Rivers, etc., Europe, Asia, Africa, North America
Periophthalmus	Normal arteries	Normal veins	Muddy shores, Africa, Asia, Australia
Boleophthalmus	Normal arteries	Normal veins	Muddy shores, East Indies and Pacific

respiratory organ is the air bladder, the exchange must be through the pneumatic duct. No observations have been made on the means by which the air is passed up the duct.

Exhaled air is passed to the exterior, often while the fish is distant from the surface, through the mouth, the gill opening, or, if the respiratory organ is the intestine, the anus.

D. Respiratory Epithelium

In the great majority of the internal organs, wherever they occur in the body, the respiratory surface is covered by a flattened epithelium, below which is a rich network of capillaries which are brought close to the contained air by thinning of the epithelial cells above them. The structure is, in fact, a direct modification of that to be expected in an unmodified internal surface. It resembles the structure of a primitive lung, and differs fundamentally from the respiratory surface of the secondary folds of the gills where the blood circulates among pillar cells in lacunar spaces without cellular walls (see p. 11). Mucus-secreting cells are often found in the epithelium of the air-breathing organs between the capillaries. More detailed accounts of the epithelia can be found in the papers by Rauther (1910), Schottle (1932), and Leiner (1938).

In the accessory organs of *Clarias*, the structure is different. These

organs are tree-like outgrowth of the dorsal ends of the gill arches and project into cavities on the dorsal sides of the gill chamber and pharynx. In them the blood circulates in very numerous parallel lacunae which contain pillar cells and resemble those of the secondary folds of the gills. The organs are believed to be derivatives of the gill arches and to owe their structure to this origin. In the labyrinthine organs of the Anabantidae (*Anabas, Macropodus, Trichogaster*) the structure of the respiratory epithelium also resembles that of the secondary folds of the gills.

In the respiratory skin of the eel (*Anguilla anguilla*), Krogh found a capillary system similar to that in the skin of the frog but with the capillaries somewhat less densely crowded. In *Periophthalmus* and *Boleophthalmus*, which also possess cutaneous respiration, the blood circulates in capillaries close to the surface of the skin which is very thin above them (Schottle, 1932).

E. Circulation (see also Chap. 2)

In general, the arterial and venous supplies of the accessory respiratory organs are modifications of those to be expected in unmodified tissues in the parts of the body where the organs occur. Thus, organs in the pharynx or gill chamber are supplied by arteries from the branchial arches and drained by veins joining the anterior cardinal or jugular veins. Those in the body cavity are supplied by the coeliac or other arteries of the gut and drain to the posterior cardinal or hepatic portal vein. The lung or air bladder, when it is respiratory, is an exception to these statements. In the Dipnoi, *Polypterus* and *Amia*, pulmonary arteries from the sixth aortic arch have been evolved. In the Dipnoi a pulmonary vein carries the blood directly back to the heart, but in *Polypterus* the lung drains to the hepatic vein, and in *Amia* to the left ductus Cuvieri. In *Lepidosteus* and in all the teleosts in which the bladder is respiratory except *Gymnarchus niloticus*, the supply is from the dorsal aorta or its branches and the return to the right posterior cardinal or hepatic portal veins. In *Gymnarchus* alone among the teleosts there is a direct pulmonary vein to the atrium, and the blood reaches the bladder by arteries from the fifth and sixth aortic arches (Assheton, 1907).

IV. PHYSIOLOGY

A. Air and Water as Respiratory Media

There are differences between respiration in air and water that should be kept in mind in discussing our subject. The most obvious of these is that air contains a much higher content of oxygen (210 cc. per liter)

than saturated water (5–10 cc. per liter). This means that less air is needed to satisfy an animal's respiratory needs.

This difference is, however, partly counterbalanced by the fact that where the organ of air breathing is internal, and the exchange with the external air is incomplete—as it always is in the fishes—the air in contact with the respiratory epithelium will contain less oxygen and more carbon dioxide than the outside air. In most cases this difference is large (p. 73, 74). In the gills, also, not all the respiratory epithelium is in contact with unaltered water of the external medium, for in some fishes it has been shown that as much as 50–80% of the contained oxygen is removed from the water of the gill current in its passage over the respiratory surface (see pp. 10–12). In the absence of any experimental data on the constitution of the alveolar air in air-breathing organs of the fishes, it is not possible to compare accurately in this respect conditions in the gills and the lungs, but both the greater oxygen content of air and the more rapid diffusion in a gaseous medium make it probable that the concentration, if not the pressure, of oxygen in the neighborhood of the respiratory epithelium is higher in normal circumstances in air-breathing organs than in the gills. Air in these organs will need renewal less frequently than water in the gill chamber.

Another difference between aquatic and aerial respiration is that the presence of bicarbonates in natural waters results in removal of free carbon dioxide diffusing into them. There is no similar process in air, and in this respect aquatic respiration has an advantage over air breathing. This advantage is increased by the fact that internal air-breathing organs, owing to the incomplete exchange with the exterior, always have some percentage of carbon dioxide in the contained air (up to 3% in fishes). We shall find that the presence of carbon dioxide in the alveolar air has other effects on the respiration when we consider the oxygenation of the hemoglobins of fish bloods in these organs (p. 75–77).

B. The Contained Gases of Accessory Respiratory Organs

Analyses of the gases in these organs, or in the air given out from them, are available for a few, but only a few, air-breathing fishes.

(1) Potter (1927) found that the air bladder of *Lepidosteus* contains less oxygen and more carbon dioxide between two inspirations than immediately after an inspiration (oxygen 7.21 and 3.79%; carbon dioxide 1.59 and 2.44%). These results show that the contained air is at all times far from atmospheric air, clearly because the exchange is incomplete. They also show that more of the carbon dioxide excretion than

of the absorption of oxygen takes place in the gills, which were also functioning.

(2) Baumert (quoted by Leiner, 1938) found 11.18–10.61% oxygen and 1.18–1.16% carbon dioxide in air released from the anus of the loach (*Misgurnus fossilis*). In specimens prevented from taking air for 6 hours he found 8.12% oxygen and 1.40% carbon dioxide, and in others which had been 8 hours without taking air he found 6.29% oxygen and 2.64% carbon dioxide. Here again most of the carbon dioxide respiration must have been through the gills.

(3) Carter and Beadle (1931) found a mean of 15.9% oxygen and 1.3% carbon dioxide in air from the intestine of *Hoplosternum litorale*, and in fish in distress from being unable to reach the surface, 0–3.4% oxygen and 3.2% carbon dioxide. In *Ancistrus anisitsi* the air of the stomach was found to contain 11.8–15.7% oxygen but no measurable carbon dioxide; and in the air bladder of *Erythrinus unitaeniatus* they found 5.8–8.7% oxygen (mean 7%) and 0.2–2.8% carbon dioxide (mean 0.7%). After 2–3 hours without air breathing the bladder contained 0–4.5% oxygen (mean 1.8%) and 0.8–2.0% carbon dioxide (mean 1.5%).

For these fishes the experimental results, together with the fact that the fish normally take air and have in some cases been shown to pass it to the accessory organ, clearly establish that the accessory organs must be accepted as true respiratory organs. The results do not determine the extent of the respiration carried on in them, but the fact that most of these fish (and many others) can live in highly deoxygenated water if allowed to breathe air shows that the accessory organs are capable of sufficient respiration to maintain the life of the fish.

The results also show that the absorption of oxygen in the air-breathing organ is much greater than the excretion of carbon dioxide, most of which must take place through the gills. This is to be expected in view of the advantages of aquatic respiration in the exchange of carbon dioxide (p. 73).

C. Air-Breathing in the Skin of the Eel (Anguilla anguilla)

Krogh (1904) experimented on the respiration of the eel both in water and air. In some of his experiments he separated the respiration of the gills and skin by placing the eel in a U-tube with mercury at the bottom of the U, the head and tail of the eel being on opposite sides of the mercury. In other experiments he prevented any respiration through the gills by closing the mouth and gill opening with sutures.

In water he found that $\frac{3}{5}$ of the oxygen uptake takes place through the skin. The carbon dioxide excretion in water is mainly through the

gills, but in air $^{10}/_{11}$ of the excretion is through the skin. In air at 16° C. almost as much oxygen is taken up when the mouth and gill opening are closed as when they are open, and the gills able to function (31.5 and 26.8 cc. per kg. per hour). At 25–26° C. the skin is able to take up only about ½ of that absorbed by a fish with open mouth and gill opening. It is concluded that the cutaneous respiration is sufficient for the life of the fish in air so long as the temperature is not above about

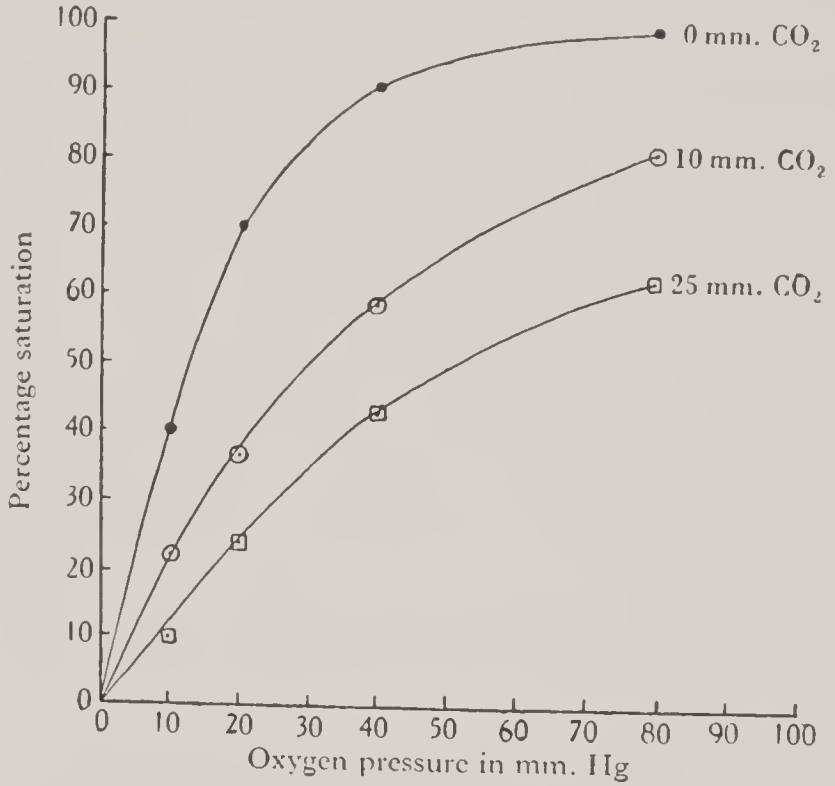

FIG. 1. Dissociation curves of blood of *Myleus setiger* (from Willmer, 1934).

15° C. This may explain why eels are usually found out of the water only at night.

Krogh also calculated the oxygen uptake per unit area of the skin and found it to be .074 cc. per sq. dm. per hour, about ½ the amount that can be taken up by frog's skin.

D. Hemoglobin and the Dissociation Curve

It has been noted that internal air-breathing organs in fishes contain carbon dioxide up to 3% of their content. Now, the oxygenation of the hemoglobin of most fishes is extremely sensitive to the presence of carbon dioxide in the medium of the blood (see p. 17), and to the lowering of pH that it causes. The sensitivity is indeed so great that 3% of carbon dioxide in the alveolar air may prevent full oxygenation of the

hemoglobin even at air pressure of oxygen. Such a blood would clearly be ineffective in an internal air-breathing organ, all the more since the pressure of oxygen in these organs is often, as we have seen, far below the atmospheric pressure. It would seem that these fish must have evolved a hemoglobin less sensitive to carbon dioxide, and indeed in this respect much more like the hemoglobins of terrestrial vertebrates, which are relatively insensitive to carbon dioxide.

The dissociation curves of some water- and air-breathing fishes of British Guiana have been investigated by Willmer (1934). In fishes of

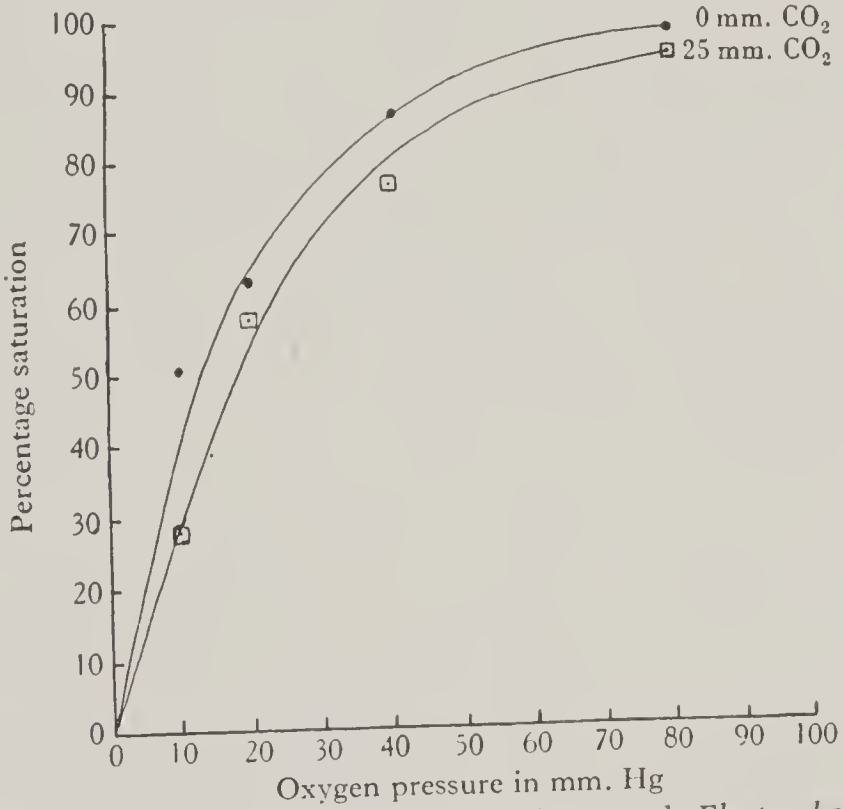

FIG. 2. Dissociation curves of blood of the electric eel, *Electrophorus electricus* (from Willmer, 1934).

the rivers, where the carbon dioxide content of the water is low (normally 2–5 mm. Hg, 0.3–0.7%), the respiration is aquatic and the dissociation curve shows a large carbon dioxide effect (Fig. 1). The blood is, in fact, of the usual fish type. Air-breathing fishes such as the electric eel (*Electrophorus electricus*) (Fig. 2) gave dissociation curves with a much smaller carbon dioxide effect, and fishes from muddy streams and pools, where the carbon dioxide content is higher than in the rivers, gave curves intermediate between these two types.

It is to be expected that other air-breathing fishes would also show a reduced carbon dioxide effect. G. R. Fish (1956) has investigated the dissociation curves of the bloods of fish from East African waters. He

found that the curves of fishes likely to be exposed to high carbon dioxide tensions showed less sensitivity to carbon dioxide than those of open-water pelagic fishes. This was also true of the air-breathing fish, *Protopterus* and *Clarias* (Fish, 1956).

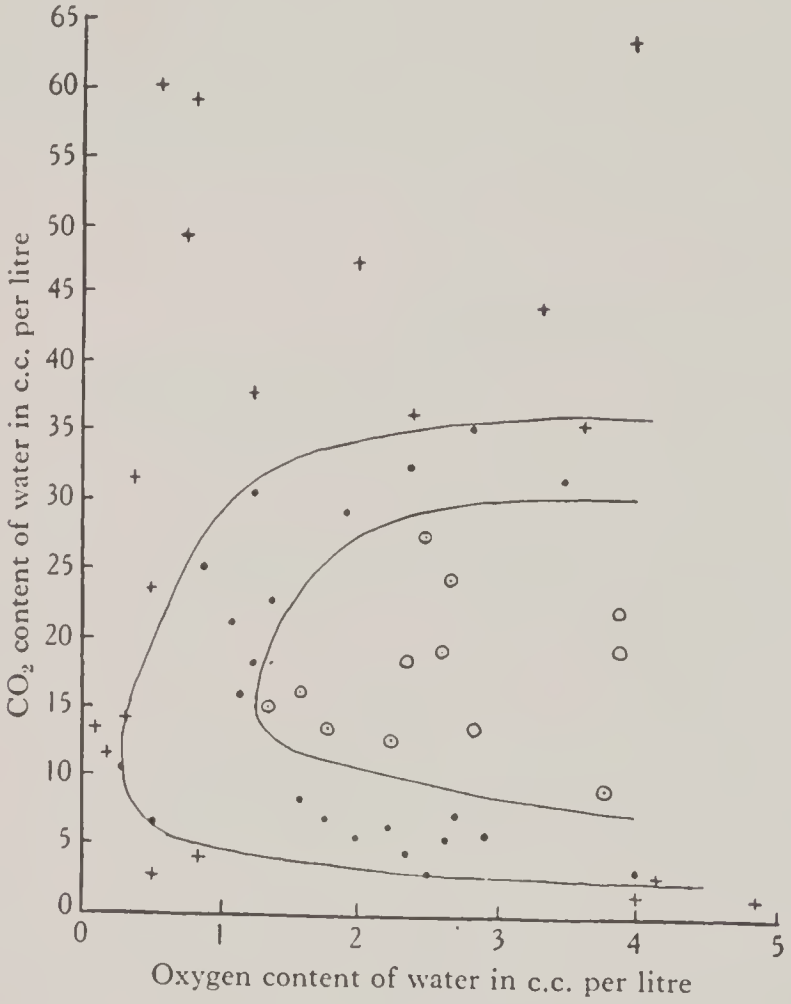

FIG. 3. Aquatic and aerial respiration in *Erythrinus unitaeniatus* (from Willmer, 1934). ⊙ = Aquatic respiration, • = Intermediate respiration, + = Aerial respiration.

E. Aquatic and Aerial Respiration in Fishes Capable of Both

The parts played by the aquatic and aerial respiration under different conditions in a fish which is capable of both have been investigated in only one species, *Erythrinus unitaeniatus* (Willmer, 1934). This fish lives in swamps in British Guiana and other parts of South America. In well-aerated water it can live by the respiration of its gills, without coming to the surface, but it has been shown to use its air bladder as an organ of aerial respiration when the water is deoxygenated (Carter and Beadle, 1931).

Willmer observed its behavior in waters with various oxygen and carbon dioxide contents, and obtained the results given in Fig. 3. Purely aquatic respiration does not occur when the oxygen content is below about 1.5 cc. per liter; the respiration is purely aerial, the gill opening being held closed, when the carbon dioxide content is above 35 cc. per liter or below about 5 cc. per liter. Between the conditions where the respiration is purely aquatic or purely aerial, there is a range in which the fish maintains the gill current and uses its gills, but also visits the surface to take air (intermediate respiration).

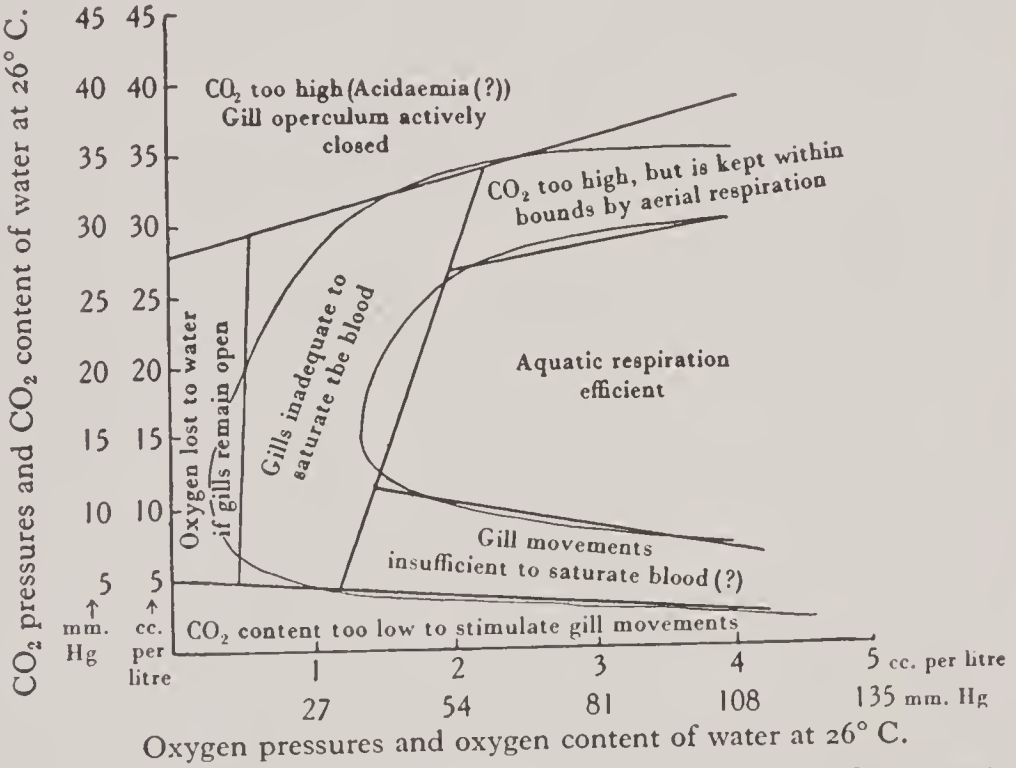
CO_2 pressures and CO_2 content of water at 26° C.
45 45
40 40
35 35
30 30
25 25
20 20
15 15
10 10
5 5
mm. Hg
cc. per litre
CO_2 too high (Acidaemia (?))
Gill operculum actively closed
CO_2 too high, but is kept within bounds by aerial respiration
Oxygen lost to water if gills remain open
Gills inadequate to saturate the blood
Aquatic respiration efficient
Gill movements insufficient to saturate blood (?)
CO_2 content too low to stimulate gill movements
1 2 3 4 5 cc. per litre
27 54 81 108 135 mm. Hg
Oxygen pressures and oxygen content of water at 26° C.

FIG. 4. Interpretation of the control of the respiration in *Erythrinus unitaeniatus* (from Willmer, 1934).

Willmer's explanation of these findings is given in Fig. 4. It is not surprising that aquatic respiration should be discontinued when the oxygen content is low or the carbon dioxide content high, but the use of aerial respiration when the carbon dioxide content is very low, even though the oxygen content may be high, needs explanation. Willmer's interpretation depends on the assumption that the respiratory center of the brain which controls the gill movements is, as in the mammals, controlled by the carbon dioxide content or pH of the blood. When the blood is too alkaline, owing to a carbon dioxide content of the water below 5 cc. per liter, the branchial respiratory muscles are not sufficiently stimulated and the gill opening is closed, the fish breathing air. This maintains the carbon dioxide content of the blood for the air bladder

contains about 0.7% carbon dioxide (equivalent to 5 cc. per liter in water at the temperature of the observations). The fact that no use is made of aquatic respiration at any carbon dioxide content so long as the oxygen content is below 0.5–1 cc. per liter is probably because use of the gills in these conditions would result in loss of oxygen from the blood to the water.

These explanations account for the facts, and are supported by other observations and arguments for which reference must be made to Willmer's paper. But they need confirmation from work on other species. At least it seems clear that the control of the double respiratory mechanism is complex and that both the carbon dioxide and oxygen contents of the water are concerned in it.

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Chapter II

THE CARDIOVASCULAR SYSTEM

J. C. Mott

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I. FRAGMENTARY NATURE OF THE AVAILABLE INFORMATION

The aquatic environment necessary to most fish presents a considerable obstacle to the adequate investigation of the circulatory system. Observations have generally been made on those species which are tolerant of laboratory conditions; these include among teleosts the common eel, *Anguilla*, and among elasmobranchs various inshore species of dogfish and skates. While there is no evidence to support the view that results obtained on such species are atypical of fish generally, it should be remembered that only a very few species have been examined of a large group of animals occupying diverse environments.

General criteria by which the physiological condition of the fish circulation may be judged barely exist, and there is, for example, little information as to the virtues and defects of various anesthetics.

As this chapter will show, there are some inescapable facts which make the investigation of the piscine circulation more difficult than that of the mammalian. It is also true, however, that modern methods of investigation have not been fully utilized.

Much of the earlier work on the physiology of the fish circulation has been reviewed by von Skramlik (1935) to whom subsequent investigators are greatly indebted.

II. ANATOMY

A. General Arrangement of the Cardiovascular System

The characteristic feature of the fish circulation is that the blood is pumped by the heart through the gills, where respiratory exchange takes place, and flows back to the heart through the systemic arteries and veins. There is thus a single pump and a single circuit of vessels. The *vis a tergo* propels the whole cardiac output first through the gill capillaries and then through the systemic capillaries; in addition a proportion of the cardiac output passes through the capillary beds of portal systems before returning to the heart.

The heart consists of four chambers arranged in series. Venous blood

enters the sinus venosus from the duetus Cuvieri and hepatie vein, and passes through the atrium, ventricle, and bulbus (teleosts), or conus (elasmobranchs), arteriosus. Thc blood delivered into the ventral aorta by the cardiac contractions thus contains little oxygen. The anatomy of the afferent branchial arteries (except in some speeies with aeeessory respiratory organs) is such that the whole of the eardiac output of poorly oxygenated blood must pass through the gills. The essential features of this arrangement are illustrated in Fig. 1. Many variations

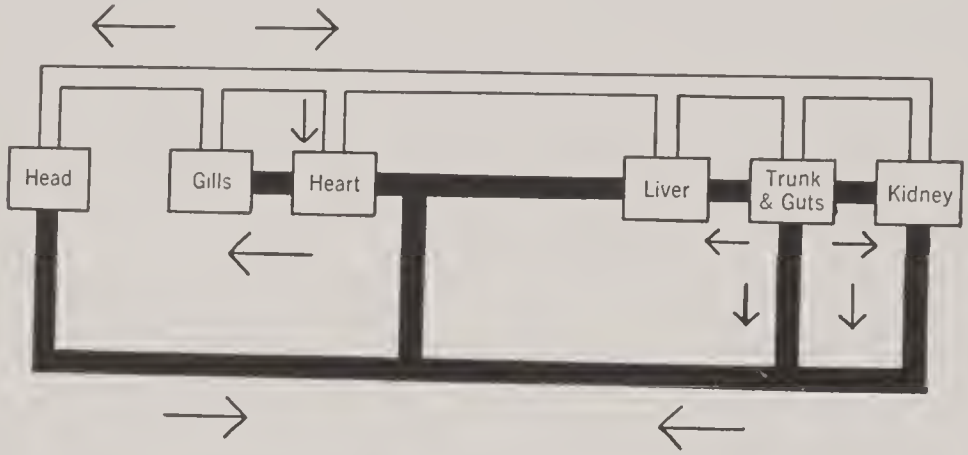

FIG. 1. Block diagram showing arrangement of the simplest type of fish circulation. Solid black vessels contain blood of lower oxygen content, white vessels blood of higher oxygen content. Arrows indicate direction of blood flow. Note coronary supply to heart muscle.

in detailed anatomy occur from species to species but these are not potentially of great physiological interest except in species with aecessory respiratory organs.

B. Valves

The typical teleost heart is provided with valves at the sinu-aurieular, auriculo-ventricular, and ventriculo-bulbar junctions. The elasmobranch heart has in addition several series of semi-lunar valves in the eonus arteriosus. Dipnoan hearts possess a curious muscular cushion of tissue instead of atrioventricular valves and a complicated system of valves in the conus arteriosus. The general function of the valves is clearly to prevent retrograde flow of blood and angiographic evidenee in the eel suggests that the valves are competent. In this fish, the anterior end of the bulbus arteriosus constricts so mueh during ventricular diastole as to suggest that this region may also have a valve-like funetion (Mott, 1950).

No valves have been demonstrated in the veins of fish. If an eel is held head upwards, its heart rapidly becomes empty of blood (von Skramlik, 1935). In the normal horizontal position, the *vis a tergo* presumably suffices to maintain the venous return to the heart.

C. The Coronary Circulation

The arterial supply of the heart in fishes [except in *Lepidosiren* (Foxon, 1950)] is derived from efferent branchial arteries and from the dorsal aorta. This well-known fact deserves to be borne in mind when considering the results of experiments on the isolated heart in which the coronary vessels are not normally preserved intact. The oxygenation of the myocardium and the distribution of substances under investigation may well be unsatisfactory in all but the smallest hearts unless the coronary arteries are themselves perfused.

III. RELATIVE DIMENSIONS OF THE CARDIOVASCULAR SYSTEM

A. Heart Weight

The hearts of fish are smaller, in relation to body weight, than those of mammals and the frog. A table of relative heart weights is given by von Skramlik (1935). The values range from 0.15% body weight for *Ophithys imberbis*, an inactive species, to 2.5% body weight for a flying fish. The comparable figure for most mammals is 4.6% and for the frog 4%.

B. Blood Volume

Table I summarizes the results of determinations of the blood volumes of several fish by various authors. The principal point of interest is that the blood volume of teleosts is generally much smaller than that of elasmobranchs which in turn is usually rather less than that of tetrapods. Martin (1950) claims that in elasmobranchs blood volume is not proportional to body weight but to (body weight)^x where $x < 1$. The averaging of his results necessary in the construction of Table I may not therefore be justifiable. He also suggests that the small blood volume of marine teleosts may be related to difficulty in maintaining their hypotonia to the environment (see Chapter IV).

C. The Red Corpuscles

The red corpuscles of fish are nucleated, and, except in the lamprey, oval in shape. Table II has been extracted from the data of Reichert and Brown (1909) and shows that fish blood contains relatively fewer

TABLE I

BLOOD VOLUME OF DIFFERENT ANIMALS EXPRESSED AS PERCENTAGE OF BODY WEIGHT

Reference	Welckar quoted by Reichert and Brown (1909)	Hyde (1908)	Derrickson and Amberson (1934)	Prosser and Weinstein (1950)	Martin (1950)		Burger and Bradley (1951)
Method	Direct Washing			Dilution Technique			
				T-1824	Vital Red	T-1824	T-1824
Cyclostomata	Lamprey 5.2						
Chondrichthyes		Small skate 4.3	*Squalus acanthias* 3.7		*Squalus sucklii* ♂ 5.2 ♀ 4.4 ♀ gr.° 11.2 *Raja* sp. 1.5 *Chimaera colliei* 6.7	♂ 14.3 ♀ 8.8 ♀ gr.° 4.7 ♂ 3.8 ♀ 4.7 ♀ 2.6	*Squalus acanthias* 6.7
Actinopterygii	"Two osseous fish" 1.4 1.9		*Tautoga onitis* 1.5	*Ameiurus natalis* 1.8	*Ophiodon elongatus* 1.9 ? *Sebastodes* 2.8 ? *Cottus* sp. 1.9 2.7	2.8	
Amphibia			*Rana catesbiana* 6.4	"Grass frogs" 8.2			
Mammalia	6.9			7.8			

° gr. = gravid.

and larger red corpuscles than that of mammals and considerably less hemoglobin. Smith, Lewis, and Kaplan (1952) recorded some degree of inverse correlation between corpuscle size and red cell count in a series of freshwater teleosts. Schlicher (1927) has published an extensive series of observations on the blood corpuscles of several teleosts under a variety of conditions, and Young (1949) noticed considerable fluctuation in the hematocrit of *Girella nigricans*, both between individuals and in single individuals examined at intervals over a period of months. The paucity of hemoglobin in fish blood reaches its limit in certain antarctic species. Ruud (1954) has reported that *Chaenocephalus aceratus*, whose habits are sluggish, has no hemoglobin in its blood.

TABLE II
COMPARISON OF NUMBER AND SIZES OF ERYTHROCYTES AND PER CENT HEMOGLOBIN IN VARIOUS ANIMALS

Animal	Erythrocytes		Per Cent Hemoglobin in Whole Blood
	Diameters in μ	Number per cu. mm.	
Lamprey	9.0	133,000	–
Skate	25.0 × 14.0	275,000–400,000	3.5 – 3.8
Torpedo	27.0 × 20.0	140,000	–
Sole	12.9 × 9.0	2,000,000	–
Tench	–	–	2.36– 3.78
Eel	14.6 × 8.9	1,100,000	–
Frog	23.1 × 14.0	404,000	2.35–10.12
Lizard	16.1 × 9.5	1,420,000	2–13
Chicken	12.2 × 7.3	3,100,000	8.5 – 9.92
Sheep	5.0	12,090,000	6.62–11.53
Man	7.9	4,886,720–5,223,205	10.4 –15.07

D. Oxygen Capacity of the Blood

Table III shows that the oxygen capacity of the blood of many fish is less than that of mammals. Species which have been reported to have blood of high capacity are generally either active (mackerel) or live in poorly oxygenated water.

E. Summary

The data assembled in this section are sufficient to indicate that an experimental approach to the piscine circulation may present difficulty on account of the relatively small size of the heart, blood volume, and

TABLE III
OXYGEN CAPACITY OF BLOOD OF FISH AND MAMMALS

Species	Habitat	Oxygen Capacity of Blood (ml. O_2/100 ml. blood)	Reference
Raia oscillata	Temperate, marine	4–6	Dill, Edwards, and Florkin (1932)
Lophius piscatorius		5.07	Root (1931)
Opsanus tau		6.21	Root (1931)
Spheroides maculatus		6.75	Root (1931)
Stenotomus chrysops		7.30	Root (1931)
Prionotus carolinus		7.66	Root (1931)
Scomber scombrus [a]		15.77	Root (1931)
Notothenia rossi marmorata	Antarctic, marine	5.99	Ruud (1954)
Notothenia coriiceps		6.24	Ruud (1954)
Chaenocephalus aceratus [b]		0.67	Ruud (1954)
Ameiurus nebulosus	Temperate, freshwater	8.4–17.4	Black (1940)
Cyprinus carpio		6.4–22.4	Black (1940)
Amia calva		8.5–13.9	Black (1940)
Catostomus commersonii		5.9–14.6	Black (1940)
Salvelinus fontinalis		11.0–13.9	Irving, Black, and Safford (1941)
Salmo trutta		11.1–14.4	Irving, Black, and Safford (1941)
Salmo gairdnerii irideus		13.0–15.0	Irving, Black, and Safford (1941)
Myleus setiger	Tropical, freshwater	10.78	Willmer (1934)
Hydrolycus scomberoides		10.22	Willmer (1934)
Pterodorus granulosus		10.60	Willmer (1934)
Hoplias malabaricus		6.53	Willmer (1934)
Hoplosternum littorale [c]		18.14	Willmer (1934)
Electrophorus electricus [c]		19.75	Willmer (1934)
Mammals		15–25	

[a] Very active.
[b] Lacks hemoglobin.
[c] Air breather.

hemoglobin content. Hall, Gray, and Lepkovsky (1926) found that asphyxia led rather rapidly to an increase of hemoglobin, red corpuscles, and other constituents of the blood. Such a phenomenon might account for the rather wide variations in oxygen capacity reported for some species.

IV. THE MYOCARDIUM

A. The Heart Beat

As in other vertebrates, the heart beat is myogenic. The heart of a 5-mm. *Scyllium* (= *Scyliorhinus*) embryo pulsates but no cardiac ganglia appear until the 13 mm. stage (His, 1893).

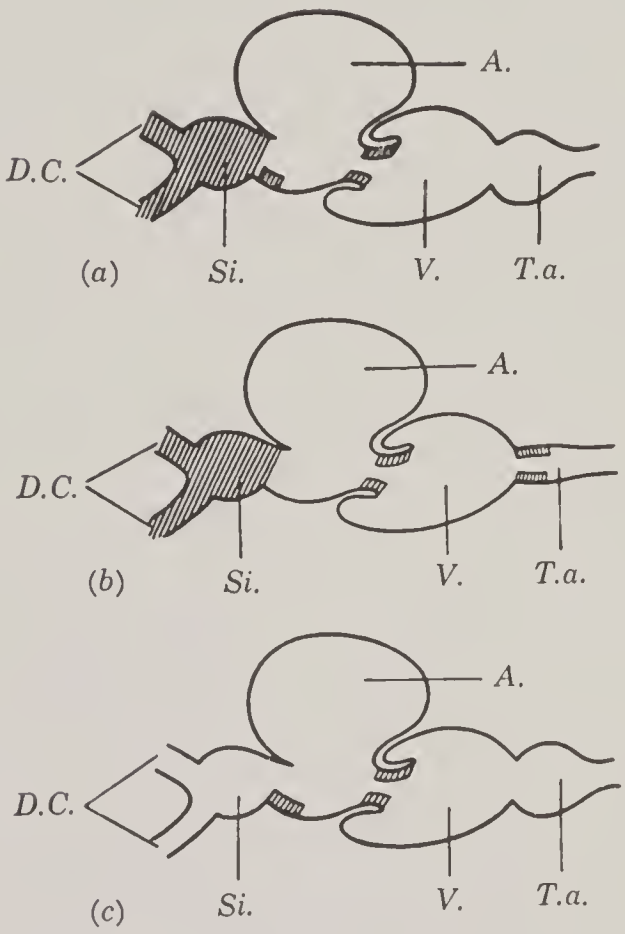

FIG. 2. Distribution of pacemakers (shaded areas) in fish hearts. a: *Anguilla* and *Conger*. b: elasmobranchs. c: teleosts other than *Anguilla* and *Conger*. D.C., ductus Cuvieri, Si., sinus venosus, A., Atrium, V., ventricle, T.a., truncus arteriosus. (From von Skramlik, 1935.)

B. The Pacemakers

Figure 2 illustrates the three groups into which von Skramlik (1935) has classified fish hearts on the basis of the distribution of the pacemakers, as determined by the classical methods of interruption of cardiac conduction by the application of ligatures, by cooling, or by stimulating electrically at appropriate parts of the heart and observing whether a compensatory pause results.

C. The Electrocardiogram

Electrocardiograms have been recorded from several species of fish including *Petromyzon* (Oets, 1950) and *Acipenser sturio* (Kisch, 1950). In the eel, Oets found the largest potential difference (about 400 μv.) with the earth electrode on the tail and the recording electrode ventral to the heart. Kisch (1948) stated that the QRS wave (ventricular contraction) was significantly shortened compared with mammals and the QT interval prolonged. The voltage on the surface of the heart was

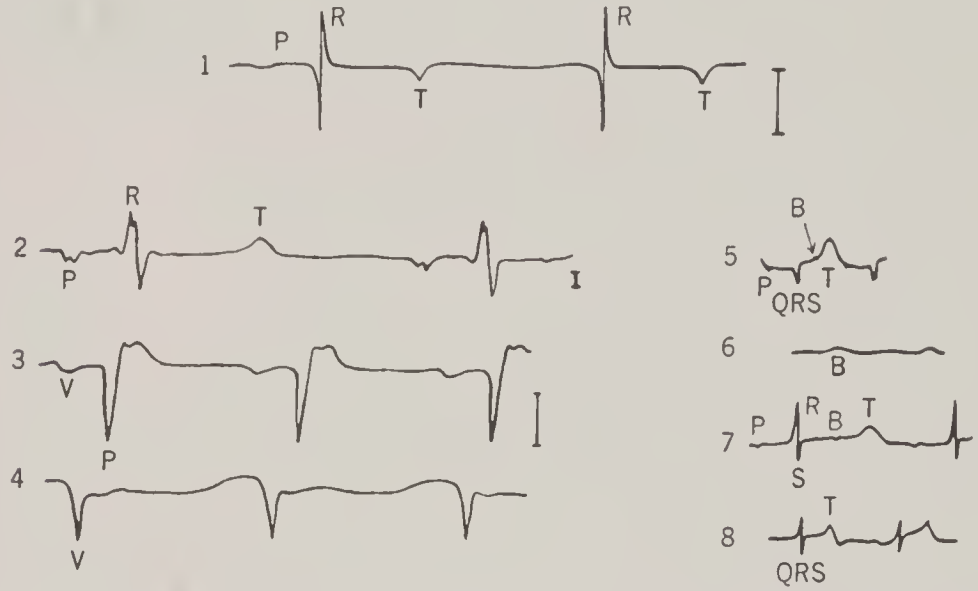

Fig. 3. Electrocardiograms of fish redrawn from Oets (1950) (1–4) and Kisch (1948, 1950) (5–8). 1: *Pleuronectes platessa,* 2: *Anguilla anguilla,* 3: isolated sinus-atrium preparation of *Anguilla anguilla,* 4: isolated sinus of *Anguilla anguilla,* 5: *Raia erinacea,* 6: isolated conus arteriosus of *Raia erinacea,* 7: bull frog for comparison, 8: *Acipenser sturio.* Corresponding points are lettered: P, represents atrial contraction; QRS, ventricular contraction; V, sinus contraction; and B, conus contraction. Vertical bars represent 100 μv.

comparable with that of a dog or rabbit. Oets (1950) showed that the P wave (atrial contraction) was preceded in the eel by a small V wave due to contraction of the sinus venosus. Kisch (1948) recorded in selachians a small deflection between the R and T waves corresponding to contraction of the conus arteriosus (Fig. 3).

V. HEMODYNAMICS

A. Cardiac Output

No determinations of the cardiac output of fish by direct methods, for example by application of the Fick principle, or by measurement of blood flow in the ventral aorta, appear to exist.

Hart (1943) compared the volumes of blood in the ventricles (of different individual fish) isolated by ligatures in systole or in diastole and computed cardiac outputs of 2.24–5.5 g./500 g. body weight/min. for 4 Canadian freshwater species. Burger and Bradley (1951) used the same and other indirect methods in *Squalus acanthias* and obtained results of similar magnitude.

A *minimum* figure for the cardiac output (in ml./kg./min) is given by the quotient $\frac{O_2 \text{ consumption (ml./kg./min)} \times 100}{O_2 \text{ capacity of blood (ml./100 ml.)}}$ providing it is assumed that the blood in the ventral aorta contains no oxygen and that the blood in the dorsal aorta is fully saturated. The data available for three fish is given in Table IV. Oxygen consumption increases with the

TABLE IV
CARDIAC OUTPUT FOR THREE SPECIES

Species	(1) Maximum Oxygen Consumption (ml./kg./min.) (Hall, 1929)	(2) Oxygen Capacity of Blood (ml. O_2/100 ml. blood) (Root, 1931)	Calculated Minimum Cardiac Output (ml./kg./min.) (1) ÷ (2) × 100
Opsanus tau	0.63	6.21	10.1
Tetraodon (Spheroides) maculatus	1.05	6.75	15.5
Stenotomus chrysops	1.00	7.30	13.7

tension, and, other things being equal, the cardiac outputs would therefore be less at lower oxygen tensions, especially for *Opsanus tau*. Little information is available on the a–v O_2 difference across the gills (see Section VI, A). However, it is unlikely to equal the capacity of the blood and the smaller it is, the greater must be the cardiac output. In the absence of direct evidence, these calculations suggest that published figures for the cardiac output may be too low.

B. Circulation Time

Angiographic evidence in the anesthetized eel indicated that the blood took 5.6 ± 1.9 seconds to pass through the gills (Mott, 1950); this is slow, when compared, for example, with the pulmonary circulation time of small mammals of 2–3 seconds. Contrast medium took at least 20 seconds (and often much longer) to pass from the heart to the visceral veins.

If the figure of 10.1 ml./kg./min. calculated for the minimum cardiac

output of *Opsanus tau* (see Section V, A) is divided into 20 ml./kg. (assuming a blood volume of 2%), the mean total circulation time in this species would be nearly 2 minutes. This time is long compared with the observations on the eel but would be reduced if the cardiac output were larger as it may well be.

C. Blood Pressure

1. The Blood Pressure Drop across the Gills

The blood pressure in the ventral aorta of fish is low by mammalian standards. Von Skramlik (1935) quotes values of from 7–10 mm. Hg in

TABLE V
Blood Pressure Drop across the Gills

Species	Mean Blood Pressure in mm. Hg		Blood Pressure Drop Across Gills in mm. Hg	Reference
	Proximal to Gills	Distal to Gills		
Torpedo sp.	16–18	6	10–12	Schoenlein and Willem (1894)
Scyliorhinus catulus	30–33	8–9	22–24	Schoenlein and Willem (1894)
Carcharias sp.	32	23.3	8.7	Lyon (1926)
Squalus acanthias [a]	28.2	15.4	16.1/4.3	Lutz and Wyman (1932a)
Squalus acanthias	39/28	30/23	9/5	Burger and Bradley (1951)
Oncorhynchus tschawytscha	74.6 [c]	53.3 [c]	21.3	Greene (1904)
Anguilla anguilla [b]	37.5	20	17.5	Mott (1950)

[a] Pithed posterior to sixth vertebra.
[b] Anesthetized.
[c] These are apparent systolic pressures on a mercury manometer; if allowance is made for the apparent pulse pressure, the mean figure for the ventral aortic pressure is about 69 mm. Hg.

Scyliorhinus canicula to a maximum of 120 mm. Hg in a Chinook salmon, *Oncorhynchus*, investigated by Greene (1904), but values of less than 50 mm. Hg are common. Table V gives the data of the pressure drop across the gills which, though variable, is always a considerable proportion of the *vis a tergo*.

Burger and Bradley (1951) in *Squalus acanthias* and Greene (1904) in the Chinook salmon found that larger fish had higher blood pressures. Lyon (1926) observed that the blood pressure in the dorsal aorta of sharks rose as high as 50 mm. Hg during struggling or swimming. The pulse pressure is less in the dorsal than in the ventral aorta as may be

seen by inspection of the records of Schoenlein and Willem (1894), Greene (1904), Lyon (1926), and Lutz and Wyman (1932a). The pressure pulse in the ventral aorta of the eel often shows a conspicuous dicrotic notch; this is presumably caused by the elasticity of the bulbus arteriosus and closure of the ventriculo-bulbar valves and offers an analogy with the conditions in the aortic arch of mammals (Mott, 1950). Rhythmical fluctuations of mean blood pressure and/or pulse pressure

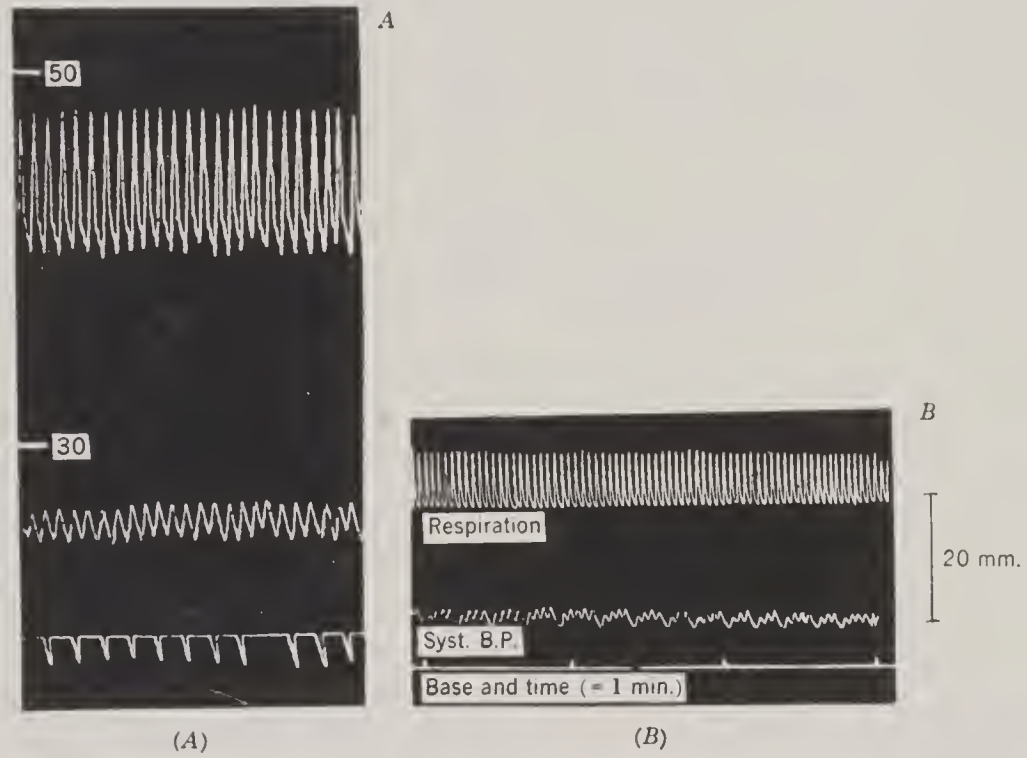

Fig. 4. A: Pressure pulse curves recorded by mercury manometers in ventral aorta (above) and dorsal aorta (below) of *Squalus acanthias*, pithed posteriorly from level of sixth vertebra. Ordinate: scale in mm. Hg; abscissa: 5–second intervals (from Lutz and Wyman, 1932a). B: Respiratory movements (above) and systemic blood pressure (below) in *Carcharias*. Note long period fluctuations on blood pressure tracing (from Lyon, 1926).

in the ventral aorta, related to interference by respiratory movements whose frequency differs from the pulse rate, have often been reported (Fig. 4).

2. Venous Pressure

It was asserted by Brunings (1899) that pressures as low as —20 cm. of water were developed in the great veins of fish by the sucking action of the heart during diastole. Schoenlein and Willem (1894) recorded pressures of —2 to —5 cm. of water in the pericardium of *Torpedo*. Such observations are a little surprising but may be possible in elasmobranchs

which have a rigid pericardium. Dohrn and Rein (1950) remark that the aspiratory action of the heart of *Scyliorhinus stellare* ceases when the pericardium is opened and cardiac efficiency falls; air emboli are said to be sucked easily into punctured veins. Schoenlein and Willem (1894) however, found a pressure of 1.3 cm. of water in a lateral cutaneous vein and of zero in a cardinal sinus of *Torpedo*. Burger and Bradley (1951) recorded zero pressure in the veins of *Squalus acanthias* except when pressor agents, e.g. adrenaline, were administered. Under such conditions negative pressures of several millimeters of mercury developed, but they do not state specifically whether the pericardium was intact or not. They found no evidence for any sucking action of the heart and did not observe negative pressures in the pericardium.

The pericardium of teleosts is not rigid though it may be tough and fibrous as in the eel in which Mott (1951) found the pressure in the sinus venosus to fluctuate between —4 and +5 mm. Hg. It is difficult to see how the maintained negative venous pressure implied by Brunings' (1899) diagram could possibly exist even in types with a rigid pericardium.

3. Summary

The foregoing paragraphs reflect the paucity of measurements of the blood pressure in different parts of the fish circulation. In quiescent fish, the mean pressure in the ventral aorta is usually less than 40 mm. Hg, the pressure drop across the gills is 10–20 mm. Hg and is possibly less in elasmobranchs than in teleosts. Venous pressures are very low. No measurements of portal venous pressures or intracardiac pressures appear to have been made. There are few records of pressure pulse curves and none taken with modern equipment of high frequency response. While we therefore have a general picture of the pressures in the different parts of the circulation, it would be highly desirable to have more measurements, especially a comprehensive set of simultaneous measurements in one species under various conditions.

VI. THE CIRCULATORY SYSTEM AND OXYGEN TRANSPORT

A. Oxygen Content of Circulating Blood

While the availability of the oxygen in the blood to the tissues is determined by the form of the relevant dissociation curve (discussed in Chap. I: Part I) the oxygen content of the blood in individual vessels is of interest in relation to the distribution of the circulation. Knowledge of the a–v difference across the gills and of the oxygen consumption would permit calculation of the cardiac output (see Section V, A) and

if the oxygen content of the blood in the "respiratory" vein and other main vessels of air-breathing fish were known it should be possible to obtain an estimate of the proportion of the cardiac output passing through the organ of aerial respiration.

TABLE VI
PERCENTAGE OXYGEN SATURATION OF THE BLOOD *in situ*

Species	No. Expts.	Condition	Percentage Oxygen Saturation of Blood in		Reference
			Ventral Aorta	Dorsal Aorta or Caudal Artery	
Trout	4		0.75	—	Ferguson and Black (1941)
Carp	3		32	—	Ferguson and Black (1941)
Sucker	2		63	—	Ferguson and Black (1941)
Catfish	1		62	—	Ferguson and Black (1941)
Raia oscillata	1	Gills perfused with aerated water	32	93	Dill, Edwards, and Florkin (1932)
Carp	2	Gills perfused with aerated water	—	85	Wastl (1928)
Tench	1	Gills perfused with aerated water	—	85	Wastl (1928)
Mackerel		Forward unobstructed swimming	—	85	Hall (1930)
		Swimming with flask attached to tail	—	46	Hall (1930)
		Stationary in screen cage	—	11	Hall (1930)

Table VI summarizes the available observations on the oxygen percentage saturation of the blood *in situ*. From these few results it seems reasonable to conclude that the blood in the dorsal aorta is normally fairly fully saturated with oxygen. Hall (1930) interpreted his results for the mackerel as indicating that this active species cannot obtain sufficient oxygen unless swimming; it is asphyxiated if prevented from moving in water in which the oxygen tension is 70 mm. Hg or less.

B. Some Limitations of Accessory Respiratory Organs

The modifications of the circulation associated with the organs of aerial respiration found in some fish have been described in Chap. I: Part II. It will suffice here to point out that in many instances the afferent

arteries of these organs are branches of the dorsal aorta and the accessory respiratory circuit is therefore in parallel with the systemic circuits (Fig. 5). It is clear that, if the environmental oxygen tension is sufficient to have saturated fully the blood emerging into the dorsal aorta from the gills, little further increase of the oxygen content of the blood passing through the accessory respiratory organ is possible. If, however, the blood in the dorsal aorta is not fully saturated, then the quantity of oxygen which can be absorbed through the accessory respiratory organ will largely depend on the proportion of the dorsal aortic blood flow traversing this organ. This proportion depends in turn on the relative vascular resistances of the systemic and accessory respiratory circuits.

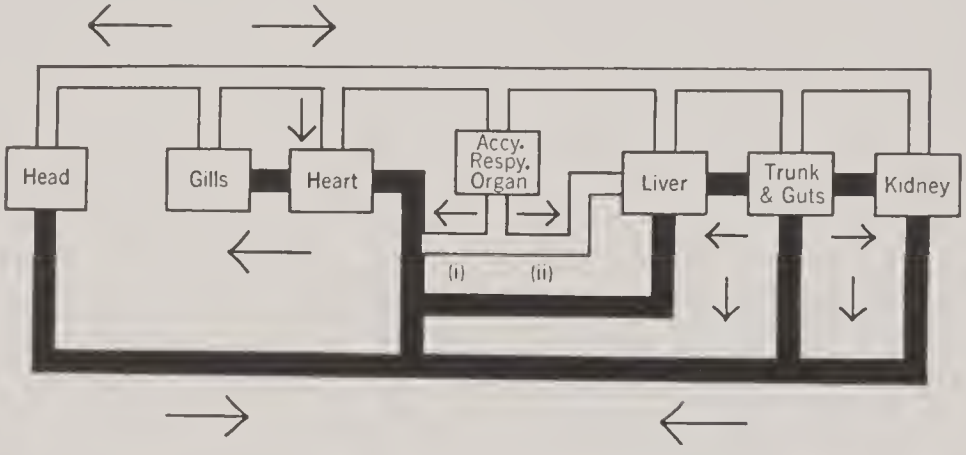

FIG. 5. Block diagram showing arrangement of the fish circulation where the circuit to the accessory respiratory organ is in parallel with the systemic circuits. The blood from the accessory respiratory organ may return direct to the heart (route i) as in Dipnoi and *Gymnarchus* or more usually (route ii) via the hepatic portal system.

Since the systemic venous and "pulmonary venous" bloods mix when they reach the heart (except perhaps in Dipnoi), if external conditions are such as to bring into use an accessory respiratory organ, it follows that under such conditions the systemic arterial blood will never attain full saturation with oxygen.

Whether the argument advanced above applies to the Dipnoi depends on the efficiency with which the complicated valvular system of the heart maintains the separation of the presumably well-oxygenated pulmonary blood entering the left atrium from the systemic venous blood entering the right atrium. The morphological arguments adduced by Boas (1880) in favor of such separation have not been tested by experiment, though Foxon (1950) reported that the blood in the anterior arterial arches of a recently dead *Lepidosiren* appeared better oxygenated than that in the posterior arches, a situation which would be consistent with the classical hypothesis.

A possible supplementary source of oxygen also exists in the gas bladder; Jones and Marshall (1953) consider, however, that, except perhaps in deep sea types, the quantity of oxygen available there is insufficient to make a significant contribution to the total oxygen requirements.

VII. THE INFLUENCE OF ENVIRONMENTAL FACTORS

A. Temperature

As in other poikilotherms, the pulse rate of the isolated or non-innervated fish heart varies directly with the temperature up to a temperature characteristic of the species, above which it starts to decline.

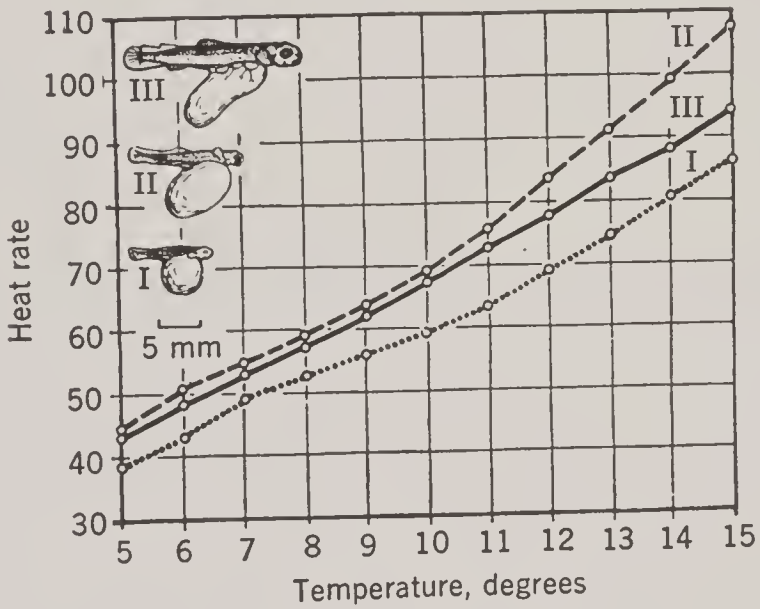

FIG. 6. The effect of temperature upon the heart rate of trout (*Salmo trutta*) embryos. I: embryos with eyeballs just pigmented, II: freshly hatched embryos, III: embryos with the yolk partly digested (from Grodzinski, 1950).

Grodzinski (1950) observed a regular increase of pulse rate in sea trout embryos from a rate of about 38 beats/minute at 5° C. to 86 beats/minute at 15° C. Isolated hearts of glass eels (*Anguilla anguilla*) had a mean frequency of 12 beats/minute at 2° C., 98 beats/minute at 25° C., and 44 beats/minute at 37° C. (Grodzinski, 1954) (Fig. 6).

Large fish have heart rates slower than those mentioned above, but the rate is still influenced by environmental temperature. Dohrn & Rein (1950) found a close correlation in intact *Scyliorhinus stellare* between the temperature of the water perfusing the gills and the heart rate, which varied from about 30 beats/minute at 10° C. to 48 beats/minute at 20° C. Burger and Bradley (1951) in *Squalus acanthias* found that

above a certain minimal value, the heart rate could vary widely in different fish, though the blood pressure of all of them was about the same. Dohrn & Rein (1950) observed that the blood pressure in the ventral aorta rose when *Scyliorhinus stellare* was cooled and fell when warmed. Conditions in experiments with intact animals are very complex and changes of temperature may cause changes in the vascular resistance of gill and systemic capillaries as well as in the activity of the myocardium. Nervous influences may also be involved.

There is no information on the relationship between pulse rate and cardiac output. It is clear, however, that the pulse rate and blood pressure of fish can be greatly influenced by change of temperature, whether directly or indirectly, and that experiments on the cardiovascular system should therefore be carried out at constant temperature.

B. Oxygen

The response of the mammalian cardiovascular system to anoxia is a rise of blood pressure and heart rate mediated through the sympathetic nervous system. There is some doubt about the role of the sympathetic nervous system in fish (see Section VIII, A), but there is a little evidence that anoxia has comparable effects on the piscine circulation. Von Skramlik (1935) mentions that an eel, asphyxiated by being removed from water, showed an increase in pulse rate of from 19 to 40 beats/minute. Dohrn and Rein (1950) found that reduction of the volume flow of water, bathing the gills of *Scyliorhinus stellare*, caused a rise in blood pressure, which was greater at higher environmental temperatures. There was little change in pulse rate but they attributed the rise of blood pressure to oxygen lack. While oxygen supply is clearly a factor requiring control during experiments on the cardiovascular system of fish, there is little information about the mechanisms of circulatory response to anoxia. However, Hall (1928) showed that asphyxia led to contraction of the spleen, and Hall *et al.* (1926) had observed hemoconcentration under these conditions.

VIII. NERVOUS CONTROL OF THE CIRCULATION

A. Innervation of the Heart

1. Vagus

Section of the cardiac branches of the vagi in fish causes an increase in heart rate (von Skramlik, 1935) and there is abundant evidence that stimulation of the peripheral end of the cardiac vagus has an inhibitory effect, abolished by atropine, on the heart. Jullien and Ripplinger

(1950a) reported that the perfusate collected from the eserinized hearts of four freshwater species of fish during vagal stimulation elicited much bigger responses from leech muscle preparations than perfusate collected without vagal stimulation. This result is consistent with the conception of the vagus as a cholinergic nerve. Jullien and Ripplinger have also claimed (1950b) that there are noncholinergic fibers in the vagus of fish, but the evidence is not conclusive.

The details of the effect of vagal stimulation on the myocardium of fish appear to be rather complex (von Skramlik, 1935) and to be related to the arrangement of the pacemakers in different types. The interest of cardiac inhibition from the point of view of the circulatory system is of course the resultant fall of blood pressure. As long ago as 1872, Jolyet described cardiac slowing and a fall of blood pressure of 50 mm. Hg in the ventral aorta of the eel during vagal stimulation.

The effects of vagal stimulation in Agnatha differ greatly from other vertebrates. In Myxinoids, no cardioregulative nerves have been demonstrated. Electrical stimulation of the medulla or of the median jugular vein of *Lampetra* caused cardio-acceleration; bradycardia was seen only after the cessation of stimulation at high frequencies (Augustinsson *et al.*, 1956).

2. Sympathetic

Though it is generally accepted that fish possess a sympathetic nervous system, there is no unequivocal evidence for any effect of the sympathetic nervous system on the heart and circulation. The inaccessibility of the sympathetic ganglia and nerves makes an experimental approach difficult.

There are, however, a few observations which prohibit a categorical dismissal of the possibility of the sympathetic nervous system playing some role in relation to the cardiovascular system.

(1) The circulatory system is not insensitive to adrenaline (a transmitter at sympathetic nerve endings in higher forms) but of course production of adrenaline in fish could be elsewhere than at nerve endings (see Section IX, B).

(2) Izquierdo (1930) obtained some evidence of increased pulse rate in *Scyliorhinus canicula* on stimulating the ductus venosus, (where he believed the cardiac sympathetic nerves run), especially in the atropinized animal, but his interpretation of his results is justifiably cautious.

(3) Atropinized fish hearts appear to respond to acetylcholine with an *increase* of pulse rate. Mott (1951) observed this in the eel; a similar effect is to be seen on records of *Scorpaena* atrium, and, even without atropinization, on the whole heart (Jullien and Ripplinger, 1950c, 1951). Such results in mammals have been interpreted as due to the stimulation

of cholinergic ganglia on the course of the sympathetic system of the myocardium (Hoffman *et al.*, 1945).

(4) Table V shows that the blood pressure of *Squalus acanthias*, particularly in the dorsal aorta, was higher in the intact than in the pithed animal. If these observations were made under comparable conditions, it is possible that the blood vessels of the intact animal are under sympathetic control.

On the other hand, Lutz (1930) failed to find any evidence for accelerator fibers to the heart in *Scyliorhinus canicula* and Burger and Bradley (1951) reported that injections of tetraethyl ammonium bromide

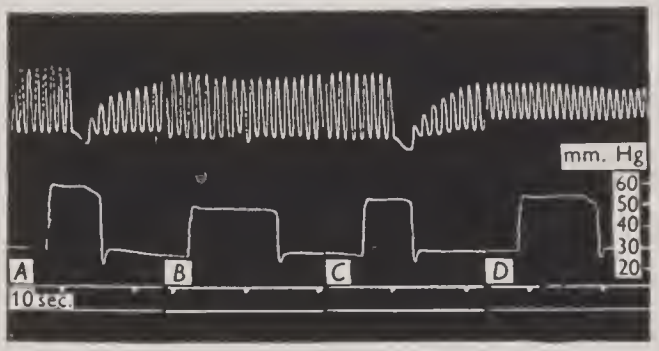

FIG. 7. Heart rate in the eel. Upper tracing is of heart rate recorded by a thread and writing lever, lower record is of the rise of pressure in the isolated first branchial arch. At A the pressure rise was adequate to inhibit the heart beat, while at B it was not. A rise of pressure which was adequate at C, had no effect at D, after the injection of 0.25 mg. of atropine between C and D (from Mott, 1951).

(which inhibits transmission at mammalian sympathetic ganglia), and of dibenamine (which blocks the action of adrenaline in mammals), had no circulatory effects in *Squalus acanthias* in doses of up to 100 mg.

While the role of the sympathetic nervous system in relation to the circulation in fish therefore remains obscure, it is possible that a systematic study of the circulatory effects of anoxia in fish might help to clarify the situation.

B. Depressor Responses

1. Reflex Inhibition of the Heart

McWilliam (1885) demonstrated in the eel that almost any sort of peripheral stimulation caused inhibition of the heart beat and this appears to be generally true in unanesthetized fish. (Wesley Mills, 1886; Greene, 1904; Lyon, 1926; Kisch, 1950). It does not normally occur in the *anesthetized* eel.

One stimulus causing cardiac slowing and fall of blood pressure is a

rise of pressure in the branchial vessels. This has been demonstrated by Lutz and Wyman (1932a) in *Squalus acanthias* and by Mott (1951) in the eel. The afferent pathway for the reflex is in the branchial divisions of the ninth and tenth cranial nerves and the efferent in the cardiac division of the vagus; this reflex is probably general in fish but it is not clear whether peripheral vasodilatation contributes to the fall of blood pressure. Since motor effects of the cardiac vagus seem difficult to elicit in Agnatha (see Section VIII, A, 1), the reflex may not exist in this group unless the efferent effects include peripheral vasodilatation (Fig. 7).

The functions which have been assigned to the branchial depressor reflex include: (1) protection of the delicate gill capillaries against high internal pressure; (2) source of normal vagal restraint; (3) reinforcement of the protective effects of spontaneous ejection of water from the pharynx (which causes a rise of blood pressure) by reducing the blood flow through the gills, thus minimizing the absorption of noxious substances. There is no evidence for or against these possibilities.

A main interest of the branchial depressor reflex in fish lies in its homology with the depressor reflexes arising from the branchiogenic zones of the arterial system of higher vertebrates (Mott, 1951).

2. Sensory Endings of the Branchial Vessels

Boyd (1936) described nerve endings in close relationship to the junction of the efferent branchial arterioles with the efferent arteries in *Mustelus* and suggested that these might provide an anatomical basis for the reflex described by Lutz and Wyman (1932a). Irving, Solandt, and Solandt (1935) recorded afferent action potentials from the cut peripheral ends of all the pre-trematic and the first four post-trematic branchial nerves in *Mustelus canis* and *Squalus acanthias*. Bursts of impulses accompanied cardiac systole; the activity was reduced by hemorrhage and increased by raising the branchial pressure or by administration of adrenaline (see Section IX, B). Mott (1951) also found that a rise of ventral aortic pressure in the eel elicited large afferent action potentials in the cut peripheral ends of branchial nerves.

In mammals the pressoreceptors of the branchiogenic areas are often closely associated with chemoreceptor endings sensitive to oxygen lack, carbon dioxide excess, and to certain drugs such as nicotine. There is no physiological evidence for such sense organs in fish and the eel is insensitive to cyanide or nicotine in less than poisonous or convulsing doses, respectively (Mott, 1951). It is possible that many experimental conditions render fish so anoxic as to preclude any further stimulation of chemoreceptors, should they exist.

C. Pressor Responses

MacKay (1931) observed in skates (*Raia diaphanes, R. erinacea, R. scabrata,* and *R. stabuliformis*) that mechanical stimulation of the skin and abdominal viscera frequently caused a moderate rise of blood pressure (measured in the ventral aorta but described as "systemic" pressure). The rise of blood pressure was often accompanied by change of heart rate and occurred in the absence of body movements. It may be significant that the average blood pressure in these large skates was only 13 mm. Hg, which is lower than that recorded by many authors and would allow pressor responses to show themselves more easily. The mechanism of any pressor response is obscure.

IX. THE REACTION OF THE PISCINE CIRCULATION TO SOME HORMONES AND DRUGS

A. Introduction

Many substances, known to be of physiological or pharmacological interest in relation to the mammalian cardiovascular system, have been tested for their effects on the fish circulation, most frequently on the isolated heart. The results have not, on the whole, shed much light on the normal physiology of the piscine cardiovascular system.

B. Adrenaline and Noradrenaline

The presence of noradrenaline and adrenaline in the chromaphil and other tissues of fish is beyond dispute (for references see Östlund, 1954). This is sufficient justification for examining the effects of these substances on the piscine circulation; moreover adrenaline injections mimic the effect of asphyxia in raising the blood sugar of fish (McCormick and MacLeod, 1925).

1. The Isolated Perfused Heart

Low concentrations of adrenaline and noradrenaline (10^{-8}–10^{-6}) cause small increases in heart rate and in amplitude of beat (Fig. 8) (Östlund, 1954; Huntsman, 1931). In higher concentrations (up to 10^{-5}), but sometimes also with the lower concentrations, the initial effect of the adrenaline is to inhibit the heart beat of elasmobranchs; the inhibition is partially prevented by atropine (Östlund, 1954). Östlund (1954) found the isolated heart of *Myxine* to be insensitive to adrenaline and noradrenaline.

2. The Gill Vessels

The gill vessels are dilated by adrenaline at concentrations of 10^{-6} in the eel (Keys and Bateman, 1932) and in the pike (Krawkow, 1913). Lutz and Wyman (1932b) found that after the injection of 4 μg. of adrenaline the percentage increase in dorsal aortic pressure was greater than the percentage increase in the ventral aortic pressure in *Squalus acanthias*, a result which is inconsistent with branchial constriction.

3. The Intact Circulation

Small doses of adrenaline (4–10 μg.) cause prolonged rises of blood pressure in elasmobranchs (Lutz and Wyman, 1932b; MacKay, 1931) and in the eel (Mott, 1951). The latter result is consistent with Keys and Bateman's (1932) observation that adrenaline 10^{-6} constricts perfused vessels of the eel's tail, though this concentration dilates the gill vessels of this species. MacKay (1931) found that no pressor response to adrenaline could be obtained in animals recently trawled. Since a second dose of adrenaline has no effect in the experimental animal unless the first dose is very small, this observation suggests that sufficient endogenous pressor amines had been liberated to preclude further adrenaline having any effect.

Burger and Bradley (1951) observed a diminution of venous pressure after the injection of adrenaline. It is not clear whether this was caused by systemic vasoconstriction or increased cardiac output.

The prolonged pressor effect of adrenaline in fish suggests that mechanisms for the inactivation of pressor amines are not conspicuous. Amine

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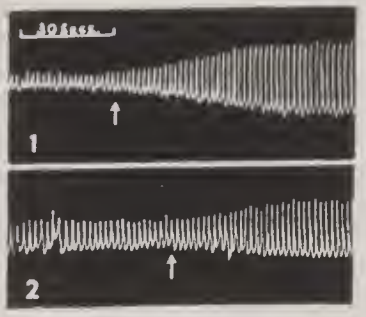
Fig. 8. Effects of adrenaline on the fish circulation. (*a*) *Squalus acanthias*, isolated heart: (1) adrenaline 1×10^{-8}, and (2) noradrenaline 5×10^{-7} introduced at arrow. (*b*): *Squalus acanthias*, isolated heart: (1) noradrenaline 1×10^{-8}, (2) acetylcholine 5×10^{-8}, and (3) and (4) adrenaline 5×10^{-9} introduced at arrow. Between (3) and (4), preparation was perfused with atropine for 3 minutes. (*c*): *Gadus callarias*, isolated heart: (1) adrenaline 6×10^{-7}, and (2) noradrenaline 1×10^{-6} added at arrow (*a–c* from Östlund, 1954). (*d*): The influence of adrenaline (2×10^{-6}) on the rate of flow through the gills of the eel, *Anguilla*, under constant pressure. The encircled points are from measurements during adrenaline perfusion and the arrows indicate times of introduction and removal of adrenaline. The continuous line shows perfusion pressure. (*e*): Same as (*d*) but perfusion of systemic vessels of posterior part of the eel at a constant pressure of 35 cm. of water (*d*) and (*e*) from Keys and Bateman, 1932). (*f*): Upper tracing ventral aortic, and lower tracing dorsal aortic blood pressure in *Squalus acanthias* pithed posteriorly from sixth vertebra. Ordinate, mm. Hg (0 for upper record = 40 mm. Hg for lower record), abscissa, time in 5 seconds. 200 μg. adrenaline injected in first and third section of record; between 1st and 2nd, 32 minute and between 2nd and 3rd sections of record, 9 minute intervals. In the fourth section of record, control injection of urea–saline in a fresh specimen (from Lutz and Wyman, 1932b).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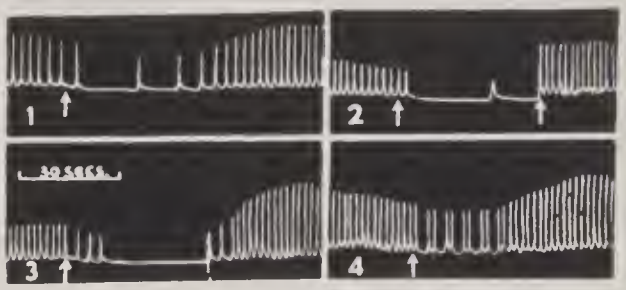
(a)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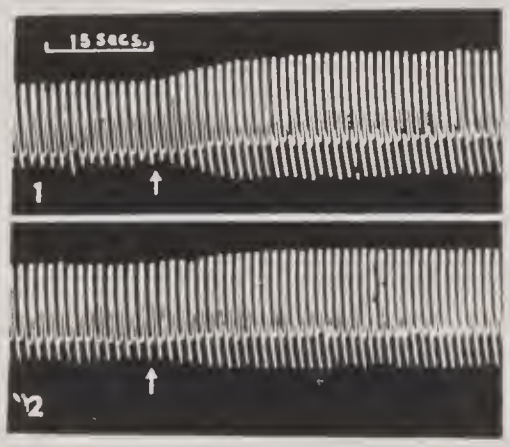
(b)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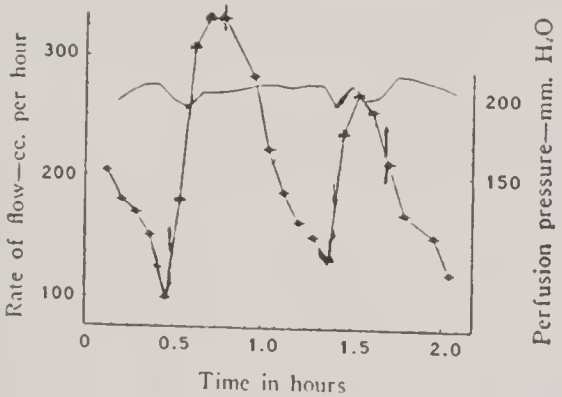
Rate of flow—cc. per hour
300
200
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0.5
1.0
1.5
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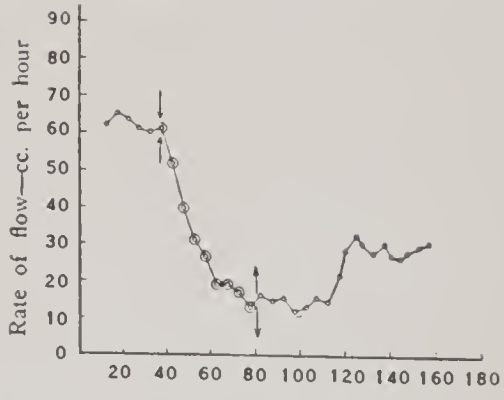
Time in hours
200
150
Perfusion pressure—mm. H₂O

(d)


Rate of flow—cc. per hour
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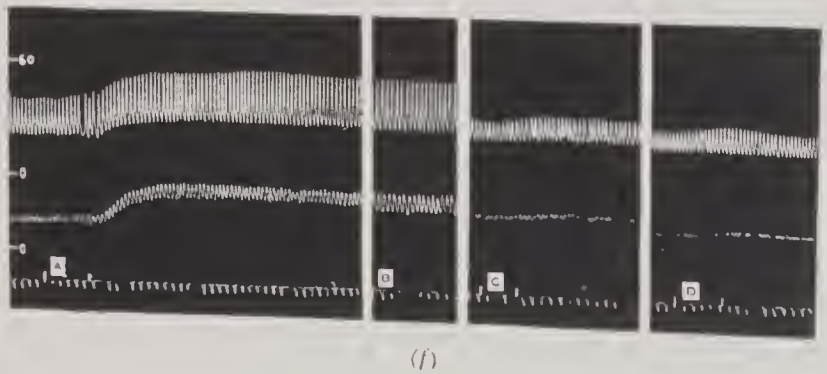
Time in minutes

(e)


60
0
A
B
C
D

(f)

oxidase occurs in fish as in all animals with chromaphil tissue (Blaschko, 1954) but this of course is not the only means by which pressor amines may be destroyed.

The effect of adrenaline on the cardiac output of fish is unknown; if the cardiac output is increased and if the diffusing capacity of the gills is adequate, adrenaline could make possible a greater uptake of oxygen by the animal.

C. Acetylcholine

Acetylcholine slows the isolated heart of *Raia diaphanes* and *R. erinacea* at a concentration of 10^{-4} (Huntsman, 1931) and in the intact eel 20 μg. also causes a fall of blood pressure (Mott, 1951). The cardiac

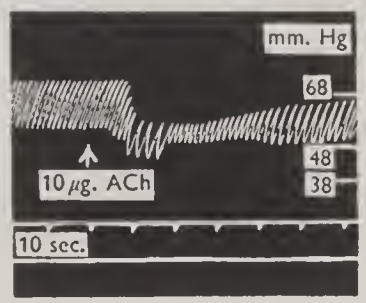


FIG. 9. Tracing of blood pressure in ventral aorta of *Anguilla* showing slowing of heart rate and fall of blood pressure caused by 10 μg. acetylcholine injected intravenously (from Mott, 1951).

inhibition is blocked by atropine. MacKay (1931) found that big doses of acetylcholine (50–400 μg./kg.), which stopped the heart of skates, subsequently led to a rise of blood pressure. Such a rise might be due to anoxia resulting from cardiac inhibition, though MacKay suggested that it was caused by the direct stimulation of some peripheral pressor mechanism, a view which is supported by the fact that atropine inhibited the cardiac slowing but only diminished the pressor effect (Fig. 9). There is no information as to the effect of acetylcholine on branchial or systemic vascular resistance.

D. Histamine

Huntsman (1931) found that the beat of the isolated skate heart was augmented and sometimes accelerated by histamine at a concentration of 10^{-6}–10^{-4}. MacKay (1931) observed only trifling effects with large doses (50 μg.-20 mg.) of histamine in skates but Mott (1951) found that 2 μg. caused a fall of blood pressure in the ventral aorta of the eel. Krawkow (1913) reported that histamine (2×10^{-6}) caused vasoconstriction in the perfused gills of the pike, *Esox*.

E. Pituitary Extracts

Pituitrin causes a prolonged rise of blood pressure in the ventral aorta of the skate (MacKay, 1931) and in the eel (Mott, 1951). Keys and Bateman (1931) found that pitressin had little effect on the branchial vessels of the eel.

F. Comment

Since vasoactive substances may have effects differing in sense or magnitude on the branchial and systemic vascular beds, which are in series, no investigation of the action of such substances in the whole animal can be satisfactorily accomplished without means of recording at least both ventral and dorsal aortic blood pressures.

X. THE LYMPH SYSTEM

Anatomical accounts of the lymph system in fishes are given by Weidenreich, Baum and Trautmann (1933), Hoyer (1938), and Przemyska-Smosarska (1951). In Agnatha and elasmobranchs the lymph system is not clearly distinct from the venous system and blood may be found in lymph vessels. Actinopterygians have lymph vessels which are more obviously differentiated from the veins. Burne (1927) described in *Lophius* and *Gadus* a system of fine "afferent" lymph vessels connected with arteries, in addition to the normal type. There are no valves in the lymph vessels of fish as is usual in cold-blooded vertebrates, which also lack lymph glands (Drinker and Yoffey, 1941), but some teleosts, e.g. eels and *Silurus,* have lymph hearts. Polimanti (1912) concluded that the lymph hearts of *Conger* were under nervous control.

The functions of piscine lymph systems are largely unknown though Wislocki (1917) found that trypan blue injected intraperitoneally in the carp, *Cyprinus,* was taken up by the lymphatic endothelium. Hoskins and Hoskins (1918) failed to demonstrate any such uptake in *Mustelus canis* and in *Acanthias.* Jourdain (1880) first described the afferent and efferent flow of lymph to be seen in the tail of young *Pleuronectes,* and it has been generally assumed that the lymph system plays a part in the transport of metabolites.

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Chapter III

THE ALIMENTARY CANAL AND DIGESTION

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I. GENERAL ORGANIZATION OF THE DIGESTIVE SYSTEM

A. Introduction

The alimentary canal of fish, like that of other vertebrates, can conveniently be regarded as divided into two main regions (Banki, 1936), the "Kopfdarm," comprising the buccal cavity and pharynx, and the

"Rumpfdarm," comprising the fore-gut (esophagus and stomach), mid-gut (intestine), and hind-gut (rectum). Further descriptive subdivision, involving the use of such terms as "duodenum," does not appear to be justified, and may, in fact, be misleading in suggesting a degree of differentiation which is not characteristic of the lower vertebrates.

Detailed descriptions of the histological organization of the alimentary system have recently been given by a number of authors (Ahsan-ul-Islam, 1949; Al-Hussaini, 1945, 1946, 1947a, 1949a, b; Blake, 1930, 1936; Curry, 1939; Dawes, 1929; Girgis, 1952a, b; Kapoor, 1953; McVay and Kaan, 1940; Rogick, 1931). The liver and pancreas call for no comment apart from the fact that the latter organ in Teleostei is commonly diffuse, and may extend over the surface of the intestine, along the portal vessels, around the gallbladder, and even into the liver itself, a situation which complicates physiological studies on that group. References to separate bile and pancreatic ducts in *Fundulus* (Babkin and Bowie, 1928) and to ducts discharging from the pancreas into the pyloric caeca (Cole and Johnstone, 1901) show that an independent pancreatic duct system sometimes persists in such cases, but it is impossible to judge whether this is generally so, since authors have not always given the necessary information. The whole alimentary canal tract itself is lined by a mucosal epithelium with which may be associated a basement membrane, a delicate layer of connective tissue (the lamina propria), and smooth muscle fibers, the latter foreshadowing the muscularis mucosae of higher forms. A layer of areolar connective tissue forms a submucosa, and in this, or in the propria, fibers may concentrate to form a more or less homogeneous layer, the stratum compactum, similar to that found in birds and mammals (Patzelt, 1936). Granular cells have been described by many authors as occurring in the wall of the alimentary canal of fish, and, indeed, of all vertebrate groups (Bolton, 1933), sometimes in such abundance as to merit the designation of "stratum granulosum" (Greene, 1912). Secretory and storage functions have been suggested for these, but, as a recent discussion shows (Al-Hussaini, 1949b), no satisfactory interpretation of them is as yet possible.

The musculature of the buccal cavity, pharynx, and esophagus is striped, and it seems to be normal for striped fibers to extend for at least a short distance over the wall of the stomach, a feature which presumably aids in the control of the swallowing and rejection of food. Over most of the stomach, however, the muscle is normally smooth, and for the rest of the alimentary tract it forms the usual muscularis, composed of inner circular and outer longitudinal fibers, although striped muscle has been reported to extend backwards to the anus in the tench (Patzelt, 1936). Between the two layers of the muscularis lie

the nerve cells and fibers of the myenteric plexus; a submucosal plexus is also present (Nicol, 1952), but not all authors have referred to this, and it could not be found in *Pleuronectes* by Dawes (1929). The alimentary canal, from the posterior end of the pharynx backwards, is delimited externally by the serosa, continuous with the mesenteries, and associated with a small amount of connective tissue which penetrates between the layers of the muscularis.

B. Evolutionary Considerations

The alimentary canal of vertebrates is seen in its simplest form in the Cyclostomata (lampreys and hagfish), and a brief reference to these is necessary in order to set the evolutionary background against which the organization of the digestive system of fish should be viewed. In the larval and adult lamprey (Barrington, 1936, 1945) a buccal cavity, pharynx, esophagus, and intestine (mid-gut) are clearly differentiated, as also is a liver, but there is no stomach. The pancreas appears to be in a very early stage of its evolution, with an intestinal caecum containing zymogen-secreting cells, closely associated with follicles which develop from the intestinal epithelium and from the bile duct and which are thought to represent the islets of Langerhans.

There is good reason for regarding the absence of the stomach from these jawless vertebrates as a primitive feature, and it has been suggested (Barrington, 1942) that its appearance in fish may have been correlated with the establishment of macrophagous feeding which was rendered possible by the evolution of jaws. The view that the stomach first arose as a storage place for ingested food is supported by the existence of intestinal swellings at the anterior end of the intestine of many stomachless fish (p. 131), one in many Cyprinidae, but two (separated by well-marked constrictions) in *Salarias* (Al-Hussaini, 1947b); these appear to function in precisely that way and in the earlier literature they are sometimes erroneously referred to as stomachs. The secretion of acid into such a sac might have arisen as an adaptation for killing the prey and for checking bacterial infection, and the production of pepsin might well have followed on this, possibly by derivation from an intracellular cathepsin (Smith, 1951). It may be assumed that early stages in the evolution of the gastric mechanism were passed through in the Placodermi, an extinct class in which the jaws appear to have been of a more primitive structure than any found in the modern groups of fish, but there is always the possibility that the digestive systems of the latter may still show primitive features and a degree of plasticity no longer persisting in the terrestrial vertebrates. At the same time it must be borne in mind that fish have followed their own lines of specializa-

tion, often with a confusing amount of parallel evolution (Young, 1950), and they may therefore have evolved features which are either unknown in higher forms, or if known, may have appeared quite independently.

C. Ecological Considerations

It is not proposed to review here the very extensive literature on the food of fish (see, for example, Suyehiro, 1941; Hartley, 1947, among recent writers), but since reference is frequently made to the "herbivorous," "carnivorous," or "omnivorous" habits of these animals it seems well to point out that many species can clearly make use of a wide range of foods; there is, in fact, much to support the view that fish will eat what they can get (Steven, 1930), and there is even evidence that under aquarium conditions some species will swallow water and utilize the bacterial and algal material suspended in it(Allee and Frank, 1948, 1949; Frank and Allee, 1950). Hartley (1948) noted that in a fish community studied by him the food chains were simple; the pike was clearly identifiable as a specialized predator, but the other members overlapped a good deal in their diet, and there was probably much competition. He concluded that a consequence of this system was that a failure of one source of food would be much less serious than if the ecological niches were better defined, and the importance of this is seen in the capacity of fish to adapt to seasonal variations in their food supply, as, for example, when a species which feeds largely on plankton in summer comes to rely much more upon fish in winter (Martin, 1954). It is clear, too, that fish can adapt in their diet to the circumstances prevailing in the particular community in which they are established. Thus Hartley (1947) found that the roach might be predominantly herbivorous or carnivorous as opportunity offered, and Hynes (1950) observed it feeding mostly on molluscs and plants, while the two species of sticklebacks which were associated with it were feeding mostly on Crustacea and insects.

Such facts suggest that the ecological situation of fish may often favor a generalized rather than a highly specialized digestive system, but the situation cannot be properly evaluated without information as to the growth rate of the particular species under varied conditions of feeding. It has been suggested (Hartley, 1947) that a carnivorous diet in roach may be associated with a higher rate of growth, and it is always possible that the organization of the alimentary system of a particular species, as, for example, in the relative concentrations of its digestive enzymes (p. 144), may be such as to obtain maximum advantage for only a limited part of the range of material which the animal is actually capable of ingesting. It must in any case be recognized that

some species are associated predominantly with particular categories of food, as, for example, *Squalus*, which in Plymouth waters feeds almost exclusively on herring, whereas *Scyliorhinus* will take anything which comes its way (Steven, 1930). Such associations must often be determined by the foraging methods of the fish concerned; *Callionymus*, for example, never normally leaves the bottom to feed and so cannot utilize megalopa larvae which may be swarming above it, while *Solea vulgaris*, which depends upon its tactile sense, differs in its diet from *Pleuronectes microcephalus* (= *Microstomus kitt*), which relies upon vision. All these considerations imply that attempts to correlate the digestive physiology of fish with their diet must be based upon extensive observations carried out under as wide a range of conditions as possible, and should give due weight to the relevant behavioral and ecological factors.

II. BUCCAL CAVITY AND PHARYNX

The buccal cavity and pharynx, which are lined by a stratified epithelium richly provided with mucous cells and often with taste buds, are concerned in the seizure, control, and probably selection of food, and may also be involved in the preparation of the food for the subsequent action of the digestive secretions, being commonly aided in these functions by a well-developed dentition. They thus constitute an important element in the organization of the alimentary tract, but it seems unlikely that they contribute significantly to the production of enzymes, although a very weak hydrolytic activity has been detected in extracts of the pharyngeal mucosa of some cyprinids (Al-Hussaini, 1949b).

The form of the teeth of elasmobranchs is very variable, but a correlation with diet is clear-cut and familiar in the modern species and can be seen also in fossils (Norman, 1947; Young, 1950). Most modern sharks are predatory fish-eaters and have sharp teeth, variable in form, but commonly bearing cusps or serrations, while the bottom-living rays have blunt or flattened teeth which present a large surface area for grinding the molluscs and Crustacea which are a major part of their diet. *Cetorhinus*, the basking shark, is exceptional in feeding upon plankton, the collection of which is effected by taking water into the mouth and straining it through the very long gill rakers. It has been calculated that during the winter months the plankton is probably insufficient in amount to meet the energy requirements of the actively feeding animal, and it is a remarkable fact that at that time the rakers are lost and the fish appears to enter upon a resting demersal stage (Parker and Boeseman, 1954).

The Holocephali stand rather apart from other living Chondrichthyes in possessing three pairs of grinding plates which are presumably formed

by the fusion of separate denticles. There is some reason for believing that this dentition was originally evolved as a mollusc-feeding adaptation in the Palaeozoic ancestors of the group (Moy-Thomas, 1939), and its presence in modern forms is said to be associated with ability to supplement an animal diet with plant material. Such a dentition must affect the mode of predation, as is suggested by Newell and Roper's (1935) report on the stomach contents of three specimens of *Chimaera;* they concluded that, in contrast to most carnivorous fish, which swallow their prey almost whole, these animals had broken up their food into small fragments and had perhaps bitten pieces out of fish while they were still alive. Such habits may have a bearing on the absence of a stomach in these animals (p. 132), and it seems worth noting that some parallel to these conditions is found in a quite unrelated group of stomachless fish, the Dipnoi (lungfish), which also possess grinding toothplates. They are described as carnivorous, but *Neoceratodus* is said to crop plants extensively, apparently in search of the animal life associated with it (Bridge, 1922).

The dentition of teleosts is commonly composed of oral teeth, found at the margins of the mouth and on the palate, and pharyngeal teeth which are especially associated with the branchial arches (Goodrich, 1930). In such predatory fish-eating animals as *Pterois* and *Belone* (Al-Hussaini, 1947b) the mouth and pharynx are beset with an armament of villiform teeth, some of which may be enlarged into canine-like tusks. The prey is often swallowed whole, without any mastication, and the teeth, sometimes supplemented by tooth-like gill rakers (Kapoor, 1953), serve primarily to prevent the escape of food material; they may be aided in this by being moveably hinged, an arrangement which allows them to be folded backwards during the actual entry of the food, while the very primitive tongue no doubt makes some contribution to the control of the prey (Suyehiro, 1941). At the opposite extreme is the complete absence of teeth as, for example, in *Hippocampus* and *Aeoliscus,* where the edentate condition is associated with planktonic feeding. In contrast to these, *Atherina,* a sublittoral plankton feeder, retains a weak villiform dentition on the jaws, the palate and in the pharynx; this has been thought to be related to the fact that during winter it takes small fish in compensation for the seasonal decline in zooplankton (Al-Hussaini, 1947a, b). The difficulty of generalizing from such data is, however, sufficiently indicated by the observation that well-developed pharyngeal teeth are an essential element of the planktonic feeding mechanism of *Tilapia esculenta* (Greenwood, 1953).

The pharyngeal dentition is often organized so as to form what is, in effect, a grinding mill, and in certain species some correlation has been

noted between the degree of development of this and the demands imposed by the nature of the food (Al-Hussaini, 1949a; Girgis, 1952a). It would, however, be a mistake to seek for too detailed a relationship of this type, for the same function can be carried out by more than one structure. It has been suggested (Suyehiro, 1941) that there is some tendency for the development of pharyngeal and oral teeth to be inversely proportional to each other, and Al-Hussaini (1947b) quotes the example of three predominantly herbivorous fish, *Acanthurus*, *Cyprinus*, and *Mugil*, the first of which comminutes its plant food with its oral teeth, the second with its pharyngeal teeth, and the third with a pylorie gizzard.

III. ESOPHAGUS

The esophagus is a short, thick-walled tube with a mucosa which, while variable, is usually composed of a stratified epithelium with cilia and mucus-secreting goblet cells, and with a strong basement membrane. Taste buds are often present, an indication, in conjunction with the presence of striped muscle, of the probable importance of this region in the selection and rejection of food. Posteriorly there is a transition to the characteristic epithelium of the digestive region of the alimentary tract, and the existence of an esophageal sphincter is often reported at the hinder end; branched papillae appear to act as valves in *Cetorhinus* (Matthews and Parker, 1950) and were found by Petersen (1908a) to be well developed also in *Squalus*, but not in other selachian genera studied by him.

IV. STOMACH

A. Morphology

Apart from the complete absence of a stomach, which will be separately discussed below (p. 131), the simplest, but not necessarily the most primitive, condition is one in which the organ appears as a spindle-shaped expansion of the posterior end of a straight fore-gut (e.g. some Gobiidae, *Esox*). Often, however, it is a curved organ (Jacobshagen, 1913; Pernkopf and Lehner, 1936), with proximal (descending) and distal (ascending) limbs. These are commonly referred to in the literature as the "cardiac" and "pylorie" regions, but this nomenclature, derived from that of the mammalian stomach, can be misleading, and it seems preferable to term these two limbs, respectively, the "corpus" or "body" and the "pyloric region" (Plenck, 1932).

There is much variation in the proportions of these parts, and, particularly in Actinopterygii, the corpus may extend backwards from the angle

as a blind sac or caecum which may assume an exaggerated size, as in *Anguilla*, where it forms the greater portion of the stomach, and in *Regalecus* (Mazza, 1900), where it actually extends into the caudal musculature. Attempts have been made to interpret such variation in terms of diet, and it has been suggested, for example, that the presence of a large caecum in active predaceous swimmers such as *Scomber* (Suyehiro, 1941) may be correlated with their capacity to attack shoals of fish and to consume large quantities of prey in a short time. No doubt the gizzard-like modification found in the gray mullet, *Mugil auratus*, is well adapted to aid in the trituration of the mud it swallows (Al-Hussaini, 1947b), but it is doubtful whether the morphology of the stomach can always be so clearly related to the nature of the diet. Considering, for example, three herbivorous forms, *Acanthurus* has a stomach with no caecum in which the distal (pyloric) limb is constricted twice before it ends at the pylorus, while in *Naseus* the stomach forms two successive loops, the proximal limb of the first loop swelling to form a ventrally directed spherical sac. In striking contrast to both of these, *Kyphosus* has a stomach with a particularly large spherical caecum (Al-Hussaini, 1947b).

B. Histology and Cytology

1. Surface Epithelium

The lining epithelium of the mammalian stomach is formed of mucus-secreting cells of a characteristic type (Babkin, 1950; Glass, 1953), which differ from true goblet cells in their shape, in the difficulty of securing from them some of the typical mucus staining reactions, and in the fact that the secretory material accumulates at their distal ends and is discharged from each cell progressively and not in a single mass. These cells (see Fig. 1A, p. 119) are believed to contribute the so-called "visible mucus" to the total gastric mucin and thus to protect the surface of the stomach from mechanical injury. It is well established that similar cells (Fig. 1A) also form the normal epithelial lining of the stomach of fish (Edinger, 1877; Kolster, 1907; Petersen, 1908b; Arcangeli, 1908; Pietruski, 1914; Dawes, 1929). In certain forms, such as *Prionotus* and *Centropristes* (Blake, 1930, 1936), in which the lining cells have been described as having at their distal ends a zone of clear cytoplasm, non-staining or difficult to stain, different methods would probably have shown them to be of the same type. A possible exception is provided by *Acipenser* (Rogosina, 1928, 1930), which is said to have typical gastric cells in the pyloric region, but typical goblet cells in the longer corpus.

Helly (1905) was the first to describe the presence of goblet cells of a peculiar type in the epithelium of the stomach, intestine, and pancreatic duct of *Torpedo*. They were characterized by the nucleus, which differed from that of the neighboring gastric epithelial cells in its larger size, spherical shape, and large nucleolus, and by their acidophil secretory inclusions, which differed from the mucoid material of typical gastric cells or goblet cells. Later similar cells were described in the gastric epithelium of certain other selachian and teleostean genera (Baecker, 1934), but there seems to be no record of their occurrence in the stomach of higher vertebrates. Similar ones, however, are found in the intestine not only of fish but of various members of all the other vertebrate groups, and Baecker has concluded that they are homologous with the Paneth cells of mammals. This will be further discussed below (p. 123), but in the meantime it may be noted that their existence in the stomach of certain fish lays open the interesting possibility that such forms may produce a wider variety of gastric enzymes than are found in the stomachs of the higher groups.

Ciliated cells have been described in the gastric mucosa of *Amia* and *Lepidosteus* by Macallum (1886), and of *Amia, Lepidosteus, Acipenser, Scaphirhynchus,* and *Polyodon* by Hopkins (1895). More recently Rogosina (1930) has confirmed their presence in the corpus in *Acipenser,* where she believed them to become transformed into the goblet mucous cells referred to above. In *Calamoichthys* (Purser, 1928) the ciliated esophageal epithelium extends a considerable distance into the morphological corpus of the stomach, and the existence of cilia in the stomach of the other forms may indicate no more than that the boundary between it and the esophagus is sometimes very ill-defined. On the other hand, it seems significant that all of the fish referred to are among the most primitive living members of the Actinopterygii, and it is thus not impossible that in their ciliated cells, in the goblet cells described by Rogosina, and perhaps in the acidophil goblet cells, they have retained features of a more primitive type of gastric epithelium (p. 111).

2. Glands

It is customary to refer to three types of gland in the mammalian stomach (Babkin, 1950). Two of these, the cardiac and pyloric glands, found respectively in the cardia (adjacent to the opening of the esophagus) and near the pylorus, are concerned only with the secretion of mucus. The chief (fundus or peptic) glands, which constitute the third type, are more extensively distributed and secrete pepsinogen and hydrochloric acid as well as mucus. They are therefore of more complex organization than are the other two and contain three types of secretory

cell, in addition to the surface epithelial cells which line the foveola or opening of all of the gastric glands. The pepsinogen is secreted by the so-called chief cells of the body of the gland, and the acid by the parietal cells which are distributed throughout the gland but which occur predominantly in the neck. A mucus secretion, believed to be a mucoprotein (Glass, 1953), is produced by a third type of cell, the mucoid neck cell, which differs in appearance and in certain staining reactions from the mucus-secreting cells of the surface epithelium. This third type, unlike the other two, is found also in the cardiac and pyloric glands, and is responsible for their mucus secretion which, in the case of the pyloric glands, probably assists in the neutralization of the acidity of the gastric juice before it enters the duodenum (Florey and Harding, 1933).

The glandular structures of the fish stomach appear to show a more primitive level of organization than the above. Yung (1899), Kolster (1907), Sullivan (1907), and Petersen (1908b) have described them in a number of selachians, but, while it is established that the chief glands (Plenck, 1932) are simple or branched tubular glands with neck and body regions, the details are not easy to correlate with more recent accounts of the mammalian mucosa. The neck possibly lacks distinctive mucoid neck cells (Yung, 1899; Sullivan, 1907; and Petersen, 1908b), implying that it is lined merely by an extension of the surface epithelium, the cells of which become modified in shape with loss of their mucus content as it extends inwards. According to Petersen the body of these glands may be lined by only one type of cell (e.g. *Squalus*) or by two (e.g. *Raja*), but little is known of the function of these beyond a general correlation between the presence of granules in some of the cells and the production of pepsin. In particular, there appears to be no evidence of any division of labor between acid- and pepsin-secreting cells.

The granular secretory cells tend to be confined to the corpus in selachians, the pyloric region being occupied by crypts which are not true pyloric glands since they are lined by cells resembling those of the surface epithelium. The boundary between these two regions is not actually a sharp one, and it is desirable in physiological studies to distinguish between the external morphological division and the internal differentiation of the mucosa which need not correspond with this (Petersen, 1908b). A cardiac region, also with crypts, can sometimes be distinguished, but it is clearly of little importance, being merely a transition zone between esophagus and stomach.

The gastric mucosa of the Actinopterygii has been studied by a number of workers (e.g. Blake, 1930) who agree that the body of the simple or branched chief glands contains only one type of granular cell which is in consequence assumed to secrete both pepsin and hydrochloric

acid. In some instances the glands are said to open by necks composed merely of the progressively modified cells of the superficial epithelium (*Box*, Arcangeli, 1908; salmon, Gulland, 1898; *Centropristes*, Blake, 1930), but in other forms there is evidence of an independent neck region formed by a different type of cell (see Fig. 1a); Pietruski (1914), in a study of seventeen teleosts, described *Serranus*, *Platichthys flesus*, and *Trachinus* as having a distinct neck region of large goblet cells, and Dawes (1929) found in *Pleuronectes platessa* a neck region of cubical mucoid cells which he considered to be comparable with the mucoid

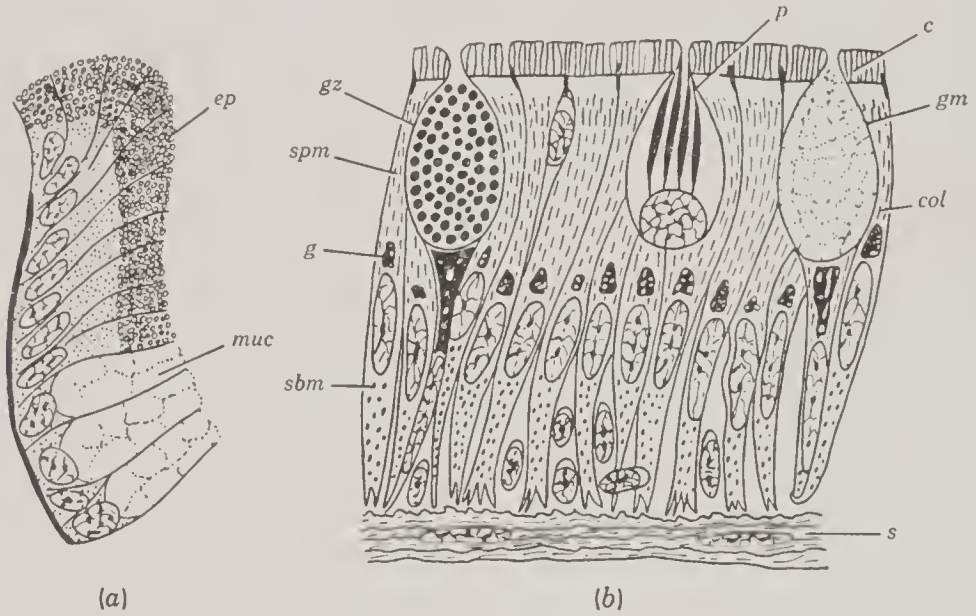

FIG. 1a. Gastric epithelial cells (*ep*) and mucus-secreting neck cells (*muc*) in a gastric gland of the perch, *Perca fluviatilis*. 1b. Intestinal epithelium of *Gobius gobio* (after Al-Hussaini, 1949b). *c*, *striated* border of columnar cell; *col*, *columnar* cell; *g*, Golgi zone; *gm*, goblet cell with mucus; goblet cell with zymogen; *p*, pear-shaped cell; *s*, stratum compactum; *sbm*, *spm*, mitochondria.

cells of the mammalian chief glands. In *Lophius* the necks of the glands are said to be formed by goblet cells with acidophil granules (Schmidt, 1915); these may correspond with Helly's acidophil cells (p. 117), although they occasionally give a positive reaction with mucicarmine.

Haus (1897) described the gastric glands as being uniformly distributed throughout the stomach of *Anarrhichas*, but in teleosts, as in the Selachii, there is a tendency for the chief glands to be confined to the corpus (Valatour, 1861; Pilliet, 1885; Herwerden, 1908; Stirling, 1884; Greene, 1912; Blake, 1930). In the salmon, however, zymogen cells have been said to extend even to the pylorus, although they are more abundant in the corpus (Gulland, 1898), and the same seems to be true of the very differently shaped stomach of *Pleuronectes* (Dawes, 1929), in which the glands become shallower in the pyloric region but do not disappear until opposite the sphincter.

V. INTESTINE (MID-GUT)

A. Morphology

The intestine (mid-gut) of vertebrates is seen in its simplest form in the Cyclostomata where it is a straight tube, the internal surface of this being increased by a longitudinal fold which follows a slightly spiral course and is commonly termed the spiral valve. The intestine preserves this pattern in the Chondrichthyes and the Dipnoi, although the spiral valve is variable and often complex in form, but in the Actinopterygii conditions become more specialized and the valve is finally lost in the Teleostei, increase of surface being then provided by growth in length of the intestine which is consequently thrown into longitudinal loops or, exceptionally, into a spiral coil. As will be seen later (p. 122) the development of blind outgrowths, the pyloric caeca, at the anterior end of the mid-gut is found in many fish, particularly among the Actinopterygii, and this is another factor increasing the effective area of the mucosal surface.

The length of the intestine in relation to that of the body varies very much from species to species, and various attempts have been made to analyze the principles determining this relationship. Klust (1939), in an extensive study of conditions within the Cyprinidae, showed that the length of the intestine increased relative to the body with age; thus, a bitterling, *Rhodeus*, with a body length of 1.1 cm. had an intestine 1.7 cm. long, giving a ratio of 1.55, while with a body length of 6.7 cm. the intestine was 14.5 cm. long, giving a ratio of 2.16. Similar observations have been made upon the carp, *Cyprinus*, by Kostomarov (1942), but it appears that in the tench, *Tinca*, which has a relatively shorter intestine than the carp, the proportions of the gut length to body length remain unaltered at all stages (Kostomarov and Pulankova, 1942). Klust's data may illustrate the operation of the familiar surface–volume relationship, lengthening of the intestine with age making some provision for the relatively greater surface area needed for secretion and absorption as the volume of the body increases. He also found, however, some effect of diet upon gut length, for a group of 4 carp fed for 3 months upon living *Daphnia* had at the end of that period an intestine/body length ratio of 2.30, while fish fed for the same period upon potato and barley had a longer intestine, giving a ratio of 2.74. The basis for this result seems to be the influence upon the intestine of the demands to which it is subjected; poor living conditions, expressed as shortage of food or limitations of space, result in reduction of the alimentary tract, while the more the latter is stimulated by use the more, within limits, does its surface increase.

Broader-based evidence for a correlation between the diet and the form of the intestine is provided by Jacobshagen (1913) who found that carnivorous teleosts (in which category he included, for example, the herring, *Clupea*, eel, *Anguilla*, stickleback, *Gasterosteus*, perch, *Perca*, and pike, *Esox*) tended to have shorter intestines (about one-third to three-quarters of the body length), while plant and mud feeders had longer ones ranging from two to five or even fifteen times the body length. Similarly, Al-Hussaini (1947b) found the intestine to be a straight tube in certain carnivores such as *Belone* and *Sphyraena*, while in herbivores it formed a complicated set of coiled loops, but, as always, it is difficult to generalize from such data, for he found a looped intestine in *Pterois*, which is a voracious carnivore. It is clear that this is a situation in which more than one factor is operative, and, as Suyehiro (1941) has pointed out, the omnivorous habits of the vast majority of fish (p. 112) must weaken any correlation between the length of the intestine and the supposedly vegetable or animal nature of the diet.

Another aspect of this problem has been raised by Al-Hussaini (1949a), who has emphasized the importance of considering the surface area of the intestinal mucosa as well as the external dimensions of the intestine. He attempted an estimate of this, using a method, developed by previous workers for use with mammals, which involved fixing and sectioning the gut after it had been inflated by saline to a known and constant pressure. Using roach, *Rutilus*, ranging in length from 95 mm. to 185 mm. he found no increase in the relative length of the gut with increase in length of the body. The estimated mucosal area of these fish was absolutely greater in the larger ones as compared with the smaller, but the ratio between the mucosal area and the total body weight ("mucosal coefficient") was less in the former, a situation explained by him on the supposition that the smaller animal is in the "growing stage" and, needing a relatively greater quantity of food, has a greater absorptive area per unit of body weight. His data for three genera of cyprinids further showed that while the relative length of the intestine was highest in *Cyprinus* (as had also been shown by Klust) and lowest in *Gobio*, the mucosal area, while following the same sequence, showed a very much smaller difference. It follows, then, that in comparisons of two species the shortness of the intestine in one may be at least partly compensated by an increase in the complexity of the mucosal pattern; even so, the longer intestine may yet have a great advantage over the shorter one under certain conditions, for it may be expected to retain food longer and this may serve as an adaptation to an herbivorous diet. It should be apparent from this discussion that the form of the intestine is the product of the interaction of a complex of factors which are not

easily analyzed, the more so since the diet of many fish must vary both quantitatively and qualitatively with the season (p. 112). The data available at present are interesting and suggestive, but there is need for a more extended analysis which can have regard to the range of variation existing within any one species.

A complicating factor in many fish is the presence of pyloric caeca (Pernkopf and Lehner, 1936). These structures, which develop as blind outgrowths of the anterior end of the intestine, are particularly characteristic of the Actinopterygii, in which group they vary greatly in number, ranging from one to over a thousand (Suyehiro, 1941; Rahimullah, 1945). Commonly they remain separate from each other, although a number may share a common opening into the intestine, but in some fish they fuse together to form a compact gland-like organ, an extreme example of this being provided by *Acipenser*. Caeca are absent from the Dipnoi and from most selachians and are said also to be absent from all stomachless teleosts (see below, p. 131), although there are many teleosts with a stomach which also lack them. It seems clear that they are of little taxonomic significance (Rahimullah, 1945) and it has been suggested that they are merely an adaptation which increases the surface area of the intestinal epithelium of fish in which the body cavity limits the length of the intestine itself or whose habits are carnivorous.

B. Histology and Cytology

In the mammalian intestine, the mucosa gives rise to characteristic glands, the crypts of Lieberkühn and Brunner's glands. Both contribute to the intestinal secretion, and the latter, which are especially found in the duodenum and resemble in certain respects the pyloric glands of the stomach, are thought to play an important part in the neutralization of the acidity of the material entering from the stomach (Florey and Harding, 1933). The situation in the lower vertebrates is simpler, and particularly so in fish, the intestine here being lined throughout by a simple columnar epithelium which, with rare exceptions (p. 124), is not elaborated into multicellular glands. This applies also to the pyloric caeca, which appear to have no cytological features clearly differentiating them from the rest of the intestine. A number of descriptions of the intestinal epithelium are available, particularly for teleosts, and, apart from some references to areas of ciliation (Ishida, 1935a), the authors concerned are largely in agreement in referring only to two main types of cell, goblet cells concerned with secretion, and columnar cells which are generally described as lacking any definite secretory inclusions and which are assumed to be concerned primarily with absorption. Too often, however, these descriptions, based on material preserved in a few routine fixatives, afford only a meager basis for any correlation

of the cytological organization of this mucosa with its supposed physiological activities, and it seems probable that much more could be learned if a greater variety of techniques was brought to bear upon it. The cytology of the columnar cells (Fig. 1B) appears to be very similar to that of the corresponding cells of the mammalian mucosa. Distally they bear a "striated border," and some authors (e.g. Dawes, 1929; Girgis, 1952b; McVay and Kaan, 1940) have observed a darkly staining and finely granular band of cytoplasm to lie between this and the basally situated nucleus. A Golgi zone lies immediately above the nucleus, and distal and basal zones of mitochondria have also been described (Al-Hussaini, 1949b). The goblet cells (Fig. 1B) are of typical shape, with a slender base and, according to Al-Hussaini, with a permanent opening through the striated border. Their mode of origin is not certainly known, although their differentiation out of the columnar cells has been claimed on the evidence of the existence of transitional stages (Blake, 1930).

Most authors give no cytological evidence for the production of enzymes in the intestinal epithelium of fish but Al-Hussaini (1949b) obtained histochemical evidence that lipase was secreted in cyprinids by the striated border cells, an observation of particular interest in view of indications, apparently unknown to him, that lipase functions as an intracellular enzyme in the carp (Schlottke, 1939). In addition, however, he found reason to believe that zymogen granules were produced in the goblet cells (Fig. 1B) throughout the length of the intestine; this did not correspond with the histochemical reaction. Proceeding on the assumption that a more intense staining of these granules was indicative of a higher concentration of zymogen, he inferred that the concentration was higher in the more carnivorous *Gobio* than in the more herbivorous *Rutilus* and *Cyprinus*, and that it was higher in the rectum and posterior intestine than in the more anterior regions. As will be seen below (p. 138), this distribution agreed with his biochemical determination of the distribution of "trypsin" in the intestines of these animals. He further found that the granules in the cells at the tips of the mucosal folds stained less deeply than those in the cells at the base, and, believing that the latter were younger than the former, he concluded that the capacity of the goblet cells to produce zymogen became less as the cells aged, while their powers of mucigen secretion persisted. These interesting conclusions need to be assessed in the light of the evidence relating to the existence of acidophil granular cells in the alimentary mucosa of vertebrates (p. 117). The only reference to such cells in teleosts seems to be in the work of Schacht (1931), who found them in the pharynx and anterior end of the intestine of the stomachless cyprinodont, *Belonesox*, but it is clearly possible that the cells described by Al-Hussaini are of the same nature.

Smallwood and Smallwood (1931) have referred to cells in the intestinal mucosa of larval carp which are perhaps identical with the pear-shaped cells (Fig. 1b) mentioned by Al-Hussaini (1949b). They contain secretory masses, resembling in appearance spireme nuclei, which break up into minute granules and pass into the lumen of the intestine; these were thought by the authors to be a digestive secretion, since they noted that the young larvae possessing such cells were able to digest *Cyclops* and *Daphnia* before the development of the pancreas. Kato (1935) has referred to the existence in *Nomeus* of so-called α cells similar to cells first described by Bühler (1930) in the stomach of *Stromateus,* but found in *Nomeus* in the stomach (except the pyloric region) as well as throughout the entire intestine and in the pyloric caeca. They were only half as high as the principal cells of the mucosa, were generally located near the lumen, and were oval in shape with a filamentous process at their base and a minute apical pore. They possessed a large, intensely stained nucleus, together with a few longitudinal striations in the cytoplasm, each composed of minute granules which Kato considered as possibly mitochondria. He offered no suggestion as to their function. Chromaffin cells, characterized by possessing basally a granulation with a positive chromaffin reaction, occur in the alimentary mucosa of many vertebrates, and have been described in the intestine of *Torpedo* and *Raja* as also in the stomach of *Acipenser* (Rogosina, 1930). They are said to be always less abundant than in higher vertebrates, and have not been reported as occurring in the Dipnoi, Holostei, or Teleostei. There appears to be no conclusive explanation of their significance (Patzelt, 1936).

The chief exception to the general absence of multicellular glands from the mid-gut mucosa of fish is provided by the Gadidae (Jacobshagen, 1937), in which invaginations develop from between the bases of the mucosal folds, and branch to form groups of small tubular glands. The cells forming the blind ends of these differ from the mucosal surface cells in their cubical shape, in the loss of the striated border, and in their marked affinity for hematoxylin. There thus seem some grounds for supposing that these structures, which occur in the mid-gut, end-gut, and also in the pyloric caeca, might produce a specific secretion, but there is no information as to the possible nature of this. Tubular glands have also been described in the intestine of *Cetorhinus* (Matthews and Parker, 1950), but no special function has been attributed to them.

VI. RECTUM (HIND-GUT)

The rectum, although usually distinguishable in fish, has not acquired in them the individuality and importance which it develops in higher vertebrates in relation to the requirements of terrestrial life. In selachians

it is lined by a stratified epithelium, in sharp contrast to the single-layered mucosa of the mid-gut, and is markedly narrower in diameter, although the thickness of the muscle, particularly of the circular layer, may be much increased. In this group, as in the Dipnoi and in *Polypterus,* there is no valve separating the mid-gut from the hind-gut (Jacobshagen, 1937), but one is often found in teleosts as the so-called ilio-caecal valve, formed by extensions of the muscles of both regions (Dawes, 1929; Al-Hussaini, 1946). In the teleosts, the hind-gut is not sharply defined externally from the mid-gut, and its presence has often been overlooked, but the existence of the valve, together with certain histological peculiarities such as an increase in the number of goblet cells and in the thickness of the muscle, justify its recognition as a region which is presumably concerned with the final expulsion of the feces. The situation in the cyprinids is less clear, for not only is the ilio-caecal valve absent, but the region said by Al-Hussaini (1949b) to be comparable with the hind-gut of other forms is characterized by a particular abundance of supposedly zymogen-secreting cells (p. 123) and it is arguable that no hind-gut is, in fact, differentiated in this group (Jacobshagen, 1937). An example of a distinctive characteristic of the hind-gut is found in the surf perches (Embiotocidae) in which group it shows a yellow, orange, or red pigmentation. This is due to xanthophylls which are derived from the shrimps eaten by these fish (Young and Fox, 1936), and which are taken up by the intestinal (mid-gut) epithelium and conveyed in the blood to the rectal (hind-gut) epithelium. There they appear to be temporarily stored until eventually excreted or, perhaps, destroyed in the blood.

The rectum of selachians bears a digitiform rectal (caecal) gland, the structure of which has been reviewed by Crofts (1925). Its function remains obscure, although the presence of a rich blood supply, together with much lymphoid tissue, has led to the view that it may be a lymph organ. On the other hand, its wall is said to contain numerous branched tubular or racemose glands which discharge a viscid secretion, and this, together with the fact that the opening of the gland is directed towards the spiral valve, has suggested that it may also be involved in digestive functions. Pixell (1908) in agreement with earlier work of Blanchard, found evidence of diastatic and emulsifying properties, but Sullivan (1907) obtained no digestive action on fibrin, starch, and ethyl butyrate; Crofts, however, using the same substrates, found some lipolytic action. Morgera (1916) believed that the secretion aided defecation by accelerating peristalsis, and in support of this view showed that extirpation or ligature of the gland prevented defecation, which could be restored by injecting extracts of the gland into the region of the posterior intestine.

VII. GASTRIC DIGESTION

A. Acidity

The acidity of the elasmobranch stomach is well marked, and Sullivan (1907) found that the free and total (i.e. free plus bound) acid might respectively reach the equivalent of 0.6% and 1% HCl. Richet long ago reported, as also did Sullivan (1907), that this acidity increased markedly after feeding, and more recently the course of events has been followed in the intact animal by Dobreff (1927), who used for this purpose the device, introduced by Weinland (1901), of removing samples of the gastric contents by means of a glass tube. Weinland had believed the gastric acidity to be due to an organic acid, but Dobreff, using the Gunzberg test, showed that free HCl was in fact present. He was able to obtain from the fasting stomach small quantities of acid mucus, and found that, even with the complete absence of food, this continued to be produced for as long as 112 days, and, in fact, until the death of the animal. During this period the acidity tended to fall, but the acid was still being secreted in detectable quantity even at the end. If a fasting animal were allowed to feed, the acidity of the gastric contents rose, but the rise was a gradual one and the maximum acidity was not attained until up to four days after the ingestion of the food, a delay ascribed by Dobreff to the initial absorption of the acid by the food mass.

The production of an acid gastric secretion is also clearly established for the Teleostei, but some authors (Herwerden, 1908; Vonk, 1927, 1929; Bayliss, 1935) have found the acidity to be much less marked than in the elasmobranchs. Bayliss has provided data for the pH of the stomach of the plaice (*Pleuronectes platessa*); in some instances he was able to obtain specimens of gastric juice, but often the stomach was empty, and he then placed drops of distilled water upon the mucosa. The pH values obtained ranged from 2.40 to 7.60, with a mean of 5.65, and he commented on the fact that these were higher than one would expect to find in a medium in which pepsin was functioning. Vonk (1929) found the pH of the gastric contents of *Esox* to have a mean value of 4.5 to 4.7, but pointed out, as did Bayliss, that the acidity might well be higher at the surface of the food mass, and the truth of this was later demonstrated (Vonk and Mennega, 1938; Vonk, 1939) by the use of the capillary glass electrode to measure the pH of prey lying within the stomach of this animal. The results showed that while the pH at the surface of a fish during its digestion in the stomach ranged from 2.4 to 3.6, this acidity rapidly declined beneath the surface to reach a value of 4.7 at a depth of only 3 mm. This explains in part why the digestion of large prey by *Esox* may take from 3 to 5 days (Vonk, 1929),

and similar conditions doubtless obtain in other fish. *Cottus, Gadus callarias,* and *G. virens* for example, may take from 5 to 6 days to digest a fish meal (Karpevitch and Bokoff, 1937), the skeleton being softened in the later period. It follows that smaller food should be digested more quickly, and this is seen in the last-mentioned animals, which can digest a meal of *Gammarus* in from 3 to 3½ days. Under such conditions the pH relationships are probably different. Thus in animals such as the cat, frog, and perch, in which the food is either divided when ingested, or is small in relation to the volume of the stomach, the situation at the beginning of digestion resembles that seen in the pike, with high acidity only at the surface of the food mass, but later, as food begins to leave the stomach, the acidity extends until the pH of the whole of the stomach contents is suitable for the action of pepsin (Vonk and Mennega, 1938; Vonk, 1939). So, also, a smaller meal may be expected to be digested more readily than a larger one, but the consequent utilization of the food is not necessarily any more efficient. The observations of Dawes (1930) on *Pleuronectes* showed that if a second meal is taken at a short interval after the first (as is more likely if the meal has been a small one) the food leaves the stomach and passes down the alimentary tract more quickly, under which circumstances digestion may well be less complete.

B. Pepsin

The identification of pepsin in the gastric contents and mucosal extracts of fish has depended upon its hydrolysis of protein substrates, such as fibrin (Vonk, 1927), gelatin (Bodansky and Rose, 1922), and casein (Bayliss, 1935) and upon the determination of its optimum pH. With regard to the latter, it should be borne in mind that while a preparation containing a single enzyme may be expected to be characterized by a pH–velocity curve with a single peak, the shape of the curve and the exact position of the peak will be affected by such factors as the presence of activators and inhibitors, and the composition of the buffers used in the digest mixtures. This has been clearly shown, for example, in the work of Norris and Mathies (1953), to be mentioned below, and it follows that crude tissue extracts, such as have often been used for the study of hydrolytic enzymes in fish, can only be expected to approximate roughly the characteristics of those enzymes. However, the results obtained from gastric preparations clearly indicate a peptic enzyme acting in the presence of hydrochloric acid. Some values quoted in the literature for its pH optimum are 2.5–2.8 for the herring (Almy, 1926), 1.65–1.8 for the perch (Hykes, Mazanec, and Szécsényi, 1934) and 1.5–2.5 for *Pleuronectes* (Bayliss, 1935). Vonk (1927, 1929, 1941),

comparing the pH optima of the pepsins of *Acanthias (Squalus), Esox,* the frog, *Testudo,* and the pig, obtained values which were all near to pH 2, and he regarded this as indicating the essential identity of the enzyme throughout the vertebrate series. He found, however, that the peptic activity of the gastric mucosa of *Esox* was much higher than that of the others, and suggested that this might be an adaptation to its lower gastric acidity. He succeeded in pursuing this characterization further than most authors, for he was able to obtain a potent preparation of the pepsin of *Acanthias,* using a method of Pekelharing which involved autodigestion, dialysis, and precipitation, and was able to compare it with swine pepsin prepared in the same way. The pH optima for the fish pepsin were 2.29 (initial) and 2.44 (final) and for the swine 2.05 and 2.41. The former proved to be five times slower (the result, in Vonk's opinion, of the presence of impurities) and 25 to 30 times weaker, but he regarded the differences as less significant than the fact that the application of the same procedure to such widely separated animals led in both cases to the preparation of highly active material with closely similar pH optima.

However, the problem of the precise identity of this enzyme throughout the vertebrate series cannot be finally settled at the level of analysis so far described. It is necessary to remember in this connection that enzymes, like other proteins, may be expected to differ, however slightly, from organ to organ or from species to species (Helferich, 1950), and these differences might conceivably provide material upon which natural selection could act to establish advantageous adaptations. That such differences do, in fact, exist seems to be clearly shown by recent studies on crystalline fish pepsin.

Norris and Elam (1940) prepared from the gastric mucosa of the Pacific king salmon (*Oncorhynchus tschawytscha*) a crystalline material, protein in its properties, which differed from Northrop's swine pepsin in the shape of the crystals and also in the details of its composition, for it contained about twice the percentage of cystine, half as much tryptophan and about seven-tenths as much tyrosine. There were also differences in temperature and pH relationships, the salmon pepsin being less responsive to temperature changes and probably maintaining its activity over a wider pH range. Using the same preparation, Fruton and Bergmann (1940) examined the specificity of salmon pepsin. In this connection, it must be remembered that the old subdivision of proteases into two categories, proteinases (hydrolyzing native proteins) and peptidases (hydrolyzing oligopeptides) is no longer maintained (Smith, 1951; Baldwin, 1952) and they are all regarded now as peptidases. The important distinction is between those which

act only upon peptide bonds joining terminal amino acid residues to the main chain (hence exopeptidases), and those which can act in addition upon more central bonds (hence endopeptidases). This latter group comprises essentially the proteinases of the older classification, including pepsin and trypsin. It has further been shown that the proteases are remarkably specific in their requirements for particular types of peptide bonds, pepsin and trypsin, for example, being able to attack protein chains only at certain points. Fruton and Bergmann were able to show, using synthetic polypeptides, that differences in specificity distinguished salmon pepsin from the pepsins of swine, cattle, sheep, and chickens; the latter, for example were able to hydrolyze carbobenzoxy-L-glutamyl-L-tyrosine and glycyl-L-glutamyl-L-tyrosine, while salmon pepsin could not.

More recently Norris and Mathies (1953) have described further differences between fish pepsins and those of warm-blooded animals. Using crystalline preparations obtained from tuna (*Thunnus albacores, T. thynnus,* and *T. germo*), they found their activity per milligram of protein nitrogen to be greater than that of any other pepsin reported. An alkali-stable form present in the gastric mucosa was assumed to be pepsinogen, and the pepsin itself was found to be more stable to alkali than was swine pepsin, a property which may perhaps be of significance in relation to the low acidity which has been reported to obtain under certain conditions in the teleostean stomach (p. 126). There was also some indication of differences in specificity between the two enzymes, tuna pepsin attacking zein only slowly, if at all, while this substance is readily split by swine pepsin.

Such results, demonstrating as they do characteristic differences between the pepsins of the different vertebrate groups, are of the greatest interest, for these differences are unlikely to be peculiar to pepsin alone. Since, however, the conditions of chemical purity under which such investigations are carried out are remote from those actually obtaining in the alimentary canal, their biological significance must be to some extent uncertain. The pH/activity curve for tuna pepsin has in fact, been shown to be greatly influenced by salts and electrolytes; two optima are obtained in the absence of salts, but only one in their presence, this lying at pH 2.5 with hemoglobin as substrate.

It has been supposed by some authors that the enzymes of poikilotherms and homoiotherms should show differences in their optimum temperatures, but the fact that the temperature optimum for crystalline tuna pepsin is at 42° C. hardly supports this view. In any case this is a complicated issue (Chesley, 1934b; Baldwin, 1952), since such an optimum is the resultant of two opposed factors, the speed of the reaction

and the thermal inactivation of the enzyme. The speed of an enzyme-catalyzed reaction decreases with time as a result of various influences, including the accumulation of reaction products and the diminution of substrate, and in order to minimize this effect it is desirable when comparing the rate of action of an enzyme at different temperatures to measure the activity for a limited period only. Unfortunately, while the results of such comparisons may be a useful basis for comparing two enzymes, their biological significance is difficult to evaluate for, apart from the complexity of enzyme-substrate relationships in the alimentary canal, the period during which the enzyme is required to act in the latter may be sufficiently prolonged for its thermolability to become a critical factor. Examples of experimental results relevant to this problem are the findings of Rakoczy (1913) and Vonk (1937) that pike pepsin is more thermolabile than that of mammals. Norris and Mathies (1953) found the same to be true for crystalline tuna pepsin, which was 50% inactivated at 56° C. after being heated for 5 minutes at pH 2, the corresponding temperature for swine pepsin being 63° C. Such a difference suggests that one of the consequences of the evolution of homoiothermy may have been the selection of greater thermostability in the enzyme systems, in adaptation to the higher temperatures at which they are normally required to operate; this possibility merits attention in future investigations of this problem. For the present it can merely be said that pepsins are characterized by differences in such properties as specificity and thermolability which might, at least in theory, be a basis for adaptive evolution.

Relating now the physiological data with the histological, there seems good evidence that the production of pepsin is associated with the granular secretory cells of the gastric glands (p. 118). This is, perhaps, most clearly seen in the selachians, where peptic activity is associated with the cardiac region (Yung, 1899; Beauvalet, 1933a; Sullivan, 1907). Less information is available for the teleosts, but Polimanti (1912), who investigated the distribution of enzymes by applying cubes of agar to the mucosa, showed that in *Box*, as in *Scyllium (Scyliorhinus) catulus*, most pepsin was found at the angle of the stomach, less at the upper end of the corpus, and least at the pyloric end. The common assumption is that pepsin and hydrochloric acid are produced in the same cells, but there seems to be no clear cytophysiological evidence for this.

C. Other Gastric Enzymes

The possibility of the production in the stomach of enzymes additional to pepsin has attracted some attention, and is of importance in view of the descriptions of anomalous secretory cells in the mucosa (p. 117).

Evidence for an amylase in the stomach of selachians has been claimed by Weinland (1901) and Beauvalet (1933a) and denied by Sullivan (1907) and Herwerden (1908). Weak amyloclastic activity was found in teleosts by Battle (1935) and Kenyon (1925), but the latter considered it to be too weak to be of significance in digestion, and Bayliss obtained negative results in *Pleuronectes*. Evidence for lipoclastic activity was found in selachians and teleosts by Polimanti (1912) and in the former by Beauvalet (1933a), but Sullivan (1907) obtained negative results for selachians as did Bayliss (1935) for *Pleuronectes*, although the latter author paid particular attention to this matter in view of Dawes' (1930) finding that absorption of fats occurred in the stomach of that animal. Finally, both Weinland and Beauvalet found evidence for tryptic action in the selachian stomach, while Ege and Obel (1935) claimed to identify chymosin (rennin) in the cod. Such positive results as have been obtained are clearly too fragmentary and uncertain to justify serious discussion, and it must always be remembered that they may indicate no more than the presence in the crude extracts of tissue enzymes, of no digestive significance, or that they may be the consequence of contamination from the intestine. On the other hand, the possibility that conditions in the fish stomach may permit of the action of enzymes other than pepsin cannot be dismissed entirely, and the problem merits further investigation, particularly in view of the evidence relating to gastric absorption (p. 149).

D. Stomachless Fish

The above data apply to what may be termed normal stomachs, but there are references in the literature to fish in which the stomach shows anomalies of structure and function. *Platichthys flesus* (Karpevitch and Bokoff, 1937) has a stomach which differs from that of *Pleuronectes platessa* in being only slightly differentiated and small in comparison with the intestine; after feeding, the pH reaches a level of 7.7, and even by the end of digestion only falls to 5.5. As an isolated case this would not be very striking, but it acquires significance if considered in conjunction with the condition reported for *Zoarces anguillaris* (MacKay, 1929a); this animal has a morphologically defined stomach, with the bile duct opening in the usual manner into the intestine below the pyloric sphincter, but the pH in fed as well as in fasting animals (tested directly on the gastric mucosa by the drop method) was near to neutrality, the readings actually extending from 6.5 to 8.4. It must be noted that the pH in these observations was recorded after the alimentary canal had been removed from animals previously killed, so that there is an obvious possibility of regurgitation of the intestinal contents into the

stomach. This was recognized by MacKay, who therefore examined other fish with a normal stomach; those in which the organ was empty gave a neutral or slightly acid reaction, but there was marked acidity when the stomach was actively digesting. Thus the lumpfish, *Cyclopterus*, and sculpin, *Myoxocephalus*, respectively, gave readings of 4.0 and 7.4 with the stomach empty, but 2.8 and 2.2 when it was full. Since some of the *Zoarces* had stomachs filled with clam or fish, MacKay concluded that there was a significant difference in that animal, and this view gained some support from her finding that extracts of its gastric mucosa had a strong lipase, possibly a very weak amylase, and only a very weak protease; the latter was not easily extractable but was presumed to be pepsin, although the gastric medium appeared not to be suited for the action of such an enzyme. Another and much more extreme example is provided by *Mugil cephalus* (Ishida, 1935b), which has a stomach lacking in gastric glands but with a gizzard-like pyloric region. The enzyme complement of this organ is said to comprise "trypsinogen" (apparently activated by an enterokinase produced in the intestine), amylase, glycogenase, maltase, and invertase, but no lipase or "erepsin." These results need confirmation and extension (there is evidence, for example, that the stomach of *Zoarces viviparus* (Kewalramani, 1953) develops a more pronounced acidity than does that of Z. *anguillaris*), but they certainly suggest that in some fish there is a tendency for a reduction of normal gastric function, and this has a bearing on what is one of the most remarkable features of the digestive organization of the whole group, the fact that in a substantial number of species the stomach is altogether lacking.

This condition obtains in the Holocephali and the Dipnoi (p. 114), but is best documented in the Teleostei, within which group it is characteristic of the Cyprinidae and Cyprinodontidae (Schacht, 1931), and is found in a number of genera scattered in other families (Hirsch, 1950). A typical and carefully studied example is provided by *Rutilus* (Al-Hussaini, 1949a, b) in which cytological examination shows a complete absence of gastric epithelium and glands, the stratified epithelium of the esophagus grading into the columnar epithelium of the intestine. The bile duct enters the latter close behind the esophagus, and biochemical tests in this and in other such forms have clearly demonstrated the absence of pepsin and of hydrochloric acid. Some earlier authors have suggested (Pietruski, 1914) that this condition is a primitive one, but it now seems far more probable that it is in fact a specialized one, resulting from the loss of the stomach present in ancestral forms (Barrington, 1942). Supporting this view is the fact that the condition occurs irregularly in unrelated groups, and that the stomach may be

present or absent in different genera of the same family (Hirsch, 1950). The close relationship of the Holocephali and Dipnoi, respectively, with the Selachii and with the terrestrial vertebrates is particularly cogent, for if the absence of a stomach from the two former groups were to be regarded as primitive it would be necessary, according to current views of phylogeny, to assume that this organ had evolved in the Selachii and in the Crossopterygii-tetrapod line independently of each other and of all other fish, a view which could hardly be considered seriously. If, on the other hand, it be accepted that the loss of the stomach has occurred independently on a number of occasions in the history of fish, the examples which have been quoted above of a possible reduction of gastric function acquire a special interest and importance.

It is difficult to point with any assurance either to the causal factors which have determined such a widespread loss of the organ, or to the advantages which may result from it. One point which can be made at once, however, is that what little is known about the control of the digestive functions of fish suggests, as will be explained below, that the pattern of nervous and hormonal influence is much less complex than in the higher vertebrates, and the complete loss of the stomach in the former might, therefore, be effected without the breaking of an elaborate chain of reactions such as would ensue in the latter. As for the mechanism by which such a loss could be brought about, some light appears to be shed on this by the condition in the larvae of amphibians. The tadpoles of the Urodela have a normally developed stomach, but in those of the Anura the development of this organ is arrested at an early stage, being recognizable by its lining of characteristic gastric epithelium but lacking in the production of pepsin and hydrochloric acid. This "larval stomach" appears to be used for compacting and storing the incoming food, and adding mucus to it from simple tubular glands, and it is not until metamorphosis that the development of the organ is completed, with the appearance of fully organized gastric glands and the production of the normal gastric secretion (Barrington, 1946; Dodd, 1950). Some analogy to this state of affairs would seem to be provided by the leptocephalus larva of the eel (Bartels, 1922). The stomach of this animal is well defined morphologically, even to the point of possessing a caecum, but it lacks secretory cells and glands, being lined by columnar cells with "cuticular borders;" glands appear at metamorphosis, but at first only in the anterior region of the caecum.

It is now well understood that such a retardation of development, often called "partial neoteny," can theoretically be produced by changes in the rates of action of the genes concerned, and the amphibians have long been known to provide classic examples of both partial and com-

plete neoteny. It is thus significant from the present point of view that the reduction of bone, which has been such a striking feature of the evolution of fish, has also been interpreted as being a neotenous phenomenon (Romer, 1946). Given, then, an alimentary canal of relatively simple organization, in a group with some tendency towards neoteny, it is possible to see factors which may have led to the reduction and loss of the stomach in fish. It is difficult, however, to relate the condition to any very clearly defined type of nutrition. Hirsch (1950) has pointed out that most stomachless fish are not predators, but use their buccal cavity and pharynx to scrape, bite off or grind their food, the fore-gut, in other words, having taken over the mechanical function of the stomach, while the intestine is now solely responsible for the chemical functions. Such facts, however, merely indicate an inevitable consequence of the loss of the stomach without explaining the origin of the condition, for, as Hirsch also points out, the reverse correlation does not hold, since by no means all of those fish which scrape and grind their food have lost their stomach. Moreover, *Scomberesox* provides an example of a predatory fish which has lost its stomach, but which swallows whole prey direct into the intestine, while *Hippocampus, Syngnathoides,* and *Aeoliscus* are examples of stomachless fish which feed largely upon planktonic Crustacea (Al-Hussaini, 1947b). It seems clear that there is no simple ecological basis for the stomachless condition, and information is certainly too limited to justify any confident assertion that it is accompanied by any clear-cut advantage to the fish concerned. For the present, it may be regarded as one example of the many features of fish organization which have evolved independently in diverse lines, and one which merits much further analysis.

VIII. INTESTINAL DIGESTION

A. pH

The pH at which intestinal digestion proceeds is necessarily the result of the interaction of the stomach contents with the secretions of the pancreas, liver, and intestine and in mammals it is known to approximate neutrality, with a tendency to vary on the acid rather than on the alkaline side. Data for fish are scanty, but as far as they go they indicate a medium approximating to neutrality with some tendency to vary towards the alkaline side.

Babkin and Bowie (1928) investigated the intestinal contents of over 50 specimens of fasting *Fundulus*. They were able to separate two or three drops of yellowish fluid from each fish while it was alive, and

obtained pH readings ranging from 8 to 9.2. The variations depended in part upon the bile; if this was present, values of 8 to 8.6 were obtained but in its absence the range was 8.8 to 9.2. In general, the reaction of the whole of the intestine was also found to be alkaline, even when food was present. The pH of the intestinal contents of *Zoarces* (MacKay, 1929a) was found to range from 6.4 to 8.4 after death, and of *Rutilus*, *Gobio*, and *Cyprinus* (Al-Hussaini, 1949b) from 6.12 to 7.72, these latter figures comparing well with values of 6.73 to 7.71 obtained by Vonk (1927) for fasted or fed *Cyprinus*. It should be remembered that *Zoarces* has a stomach with reduced acidity (p. 131) and that *Fundulus* and the three cyprinids have no stomach at all, but results from fish with a normal stomach are often not markedly different. Thus MacKay, in the course of her work on *Zoarces*, studied also several other genera, including both rays and teleosts, and obtained pH values for their intestinal contents of 6.7 to 8.4 with the stomach empty, and 4.6 to 7.4 with it full, the 4.6 reading being given by a ray with a partially digested herring in its stomach. Bayliss (1935), using the quinhydrone electrode, found that the intestinal contents of *Pleuronectes platessa*, which usually consisted of a clear watery fluid with a certain amount of fecal masses or food, were decidedly alkaline, giving pH values ranging from 7.43 to 8.65. He further demonstrated the capacity of the intestine to neutralize acid by inserting into it 1.5 ml. of $N/10$ hydrochloric acid, and then, after half an hour, titrating the contents with $N/5$ sodium hydroxide. He found 1.5 ml. of the acid had disappeared, either by neutralization or by absorption, but found it impossible to say whether the result was a consequence of true secretion of base or of ionic interchange.

Data relative to the pancreatic juice and the bile are also scarce. Babkin (1929) obtained samples of both fluids from *Raja*, and found the former to have a pH of 6.6 to 7.2, being somewhat less alkaline than that of the dog. The hepatic bile gave values of 7.5 to 7.6, while bile from the gall bladder was more acid, with a pH of 6.4. Other authors have also found fish bile to be slightly acid, values of 5.5 having been given for the gall bladder bile of *Cyprinus*, 6.8 to 7.2 for *Fundulus*, 5.4 to 6.2 for *Zoarces*, and 5.4 to 6.8 for *Raja*, with one specimen of the latter giving a reading of 7.6 (Vonk, 1927; Babkin and Bowie, 1928; MacKay, 1929b). Values given by Bayliss (1935) for the bile of *Pleuronectes* are slightly more alkaline, ranging from 7.25 to 8.05, with one at 5.75. MacKay compared her figures with similar data for mammals, quoting 6.39 to 6.74 for the dog and 7 to 7.5 for the sheep and rabbit as examples of these, and concluded that the bile in fish was somewhat more acid than in that group. Babkin, reviewing his own data, drew attention to the fact that the pancreas in

Raja constituted only about 1/800th of the total body weight, as compared with 1/400th in the dog, and suggested that the alkalinity of the intestine in elasmobranchs might be due mainly to the intestinal secretion. The data are not adequate for establishing whether or not this suggestion is correct, and the most that can be said is that the intestinal pH is not very different from that of mammals but that the interrelationship of the factors responsible for this is not clear.

B. Proteases

Much of the work on the proteolytic phase of intestinal digestion refers to the enzymes which were formerly known as "trypsin" and "erepsin," and it will be convenient to deal with this work first. Sullivan (1907) studied elasmobranchs, including *Mustelus, Carcharias littoralis,* and *Squalus* and found that extracts of the pancreas contained "trypsin," usually in an inactive form which could be activated by extracts of the intestine; the latter had no digestive activity itself, but was presumably producing the activating enterokinase which is a well-established feature of mammalian digestion, and which was not, incidentally, present either in stomach or in rectal gland extracts. Babkin (1929) succeeded in obtaining pancreatic juice from *Raja,* and, using this as well as extracts of the organ, secured results which were in essential agreement with Sullivan's; a protease was present in both juice and extract, but required activation by extracts of the mucosa of the upper intestine or of the spiral valve region, the former having the greater effect. In strong contrast to these results, Beauvalet (1933a) claimed to have confirmed the early work of Richet, to the effect that the pancreas of *Scyllium (Scyliorhinus)* could not digest protein, by showing that extracts of this organ had no effect on fibrin whereas the liver and bile digested it to peptones. It seems possible that the pancreatic extracts in this work may have needed activation, and that the liver effect was merely a demonstration of catheptic action, but it is clear, in the light of the work of Sullivan and Babkin, that these negative results cannot be accepted without confirmation. The existence of "erepsin" in the intestine of *Scyllium (Scyliorhinus)* was demonstrated by Polimanti (1912), who showed that this enzyme and enterokinase were most abundant at the anterior end and diminished down the length of the gut.

As mentioned earlier, the situation in the teleosts is frequently complicated by the diffuse form of the pancreas in that group, and by the fact that it may sometimes invest the gall bladder and extend into the liver. This occurs, for example, in *Fundulus* and accounts for the results reported by Babkin and Bowie (1928), who found that the liver and

bile of this fish showed a "tryptic" action. They recognized, after subsequent histological study, that the enzyme might have originated in the pancreas, and this was subsequently shown to be so by MacKay (1929b), who found that if the pancreatic tissue around the gall bladder was fixed by dipping the whole organ in Bouin's fluid for 1 minute before removing the bile, the latter could be obtained free of any enzyme activity. Failure to adopt this precaution may account for Chesley's (1934a) detection of enzymes in the bile of various teleosts. In the experiments of Babkin and Bowie no digestion of fibrin was effected by bile alone at pH 8.3, nor by the intestinal extracts alone (even after 62 hours) at pH 2, 8, 8.4, or 9. If, however, 1 drop of fresh intestinal extract was added to a digest mixture containing 2 ml. of bile and 8 ml. of water, digestion was completed in 3½ hours. They therefore concluded that this (pancreatic) enzyme required activation by an intestinal enterokinase. A very similar situation exists in *Pleuronectes,* which has been thoroughly studied by Bayliss (1935). Using peptone as substrate, he found "erepsin" in relatively large quantities in all parts of the intestine, with an optimum pH of 7.5 to 8. "Tryptic" activity was also found, using casein as a substrate, with an optimum of 7.5 to 8.5, these figures, together with those for "erepsin," agreeing well with the values obtained for the pH of the intestinal contents (see above, p. 135). In this animal the pancreas also extends into the liver, as well as closely investing the pyloric caeca, and hence glycerol extracts of the liver showed marked proteolytic activity. This was in part due to cathepsin which, with an optimum pH of 5.5 to 6.0, predominated in the absence of intestinal activation; with the addition of intestinal extracts, however, the "trypsinogen" was activated and the optimum pH of the whole extract shifted to pH 7. Bile obtained by cannulation or, following MacKay's procedure, after immersion of the investing pancreatic tissue for 5 seconds in Zenker's fluid, showed no proteolytic activity, but if it was allowed to extract the gall bladder for 1–2 days a marked activity developed which was much increased by intestinal activation. That this was derived from the pancreas and not from the liver cathepsin was indicated by the optimum pH, which ranged from 7.5 to 8.5. Bayliss attempted to determine whether the pancreas was the sole source of "trypsin," or whether some production also occurred in the intestine, and to this end compared the activity of caecal extracts (which also included the investing pancreatic tissue) with extracts of the more posterior intestine which could be freed from the pancreas. If the situation were as in mammals (see below), with the pancreas as the sole source of the enzyme, it would be expected that the former extracts would be markedly more active than the latter, but unfortunately,

although this was found to be so in some experiments, the results as a whole were discordant and the source of the enzyme could not therefore be defined.

In general agreement with the above are the results of Beauvalet (1933b), who found "tryptic" activity increased by intestinal extracts in the combined liver and pancreas of *Tinca, Cyprinus, Ameiurus,* and *Micropterus;* of Oya *et al.* (1927), who found a "trypsin" with a pH optimum of 7 to 8 in the pancreas of *Anguilla;* and of Oya and Yokota (1933) who found in the catfish, *Parasilurus,* a "trypsin" with an optimum at pH 7.7, present in the pancreas mostly as "trypsinogen" which was activated by the intestinal mucosa. Ishida (1936) also found "tryptic" activity in the combined liver and pancreas of four stomachless teleosts, with an enterokinase in the intestine, while Polimanti (1912), using the agar method already mentioned (p. 130), found that in *Conger* and *Box,* as in *Scyllium (Scyliorhinus),* "erepsin" and enterokinase were both present in the intestinal mucosa and most abundant at the anterior end. MacKay (1929a) was unable to detect any protease in extracts of the liver and intestine of *Zoarces,* but found a very strong one, with an optimum pH range of 6.4 to 8.2, in the contents of the stomach. This was additional to the very weak peptic type of protease in the gastric mucosa (see above p. 132) and since there was no evidence of its production either in the stomach or in the intestine, she concluded that it originated in the pancreatic secretion.

The available data seem to show reasonably clearly that "erepsin" and enterokinase are probably formed exclusively in the intestine of fish, and that "trypsinogen" is commonly, and perhaps always, formed in the pancreas, but doubt remains as to how far, if at all, the latter is also secreted by the intestine. By analogy with mammals this would not be expected, and this is the conclusion suggested by most of the data, but an exception is provided by the recent careful study by Al-Hussaini (1949b) who found, using casein as substrate, that the intestines of the cyprinids *Cyprinus, Rutilus,* and *Gobio* showed proteolytic activity which, remarkably enough, was highest in the posterior third of the tract (p. 123). He interpreted this as supporting the view of Beauvalet that while in fish with a stomach the secretions of the intestine do not affect proteins to any appreciable degree (this presumably ignores the existence of "erepsin"), the loss of the stomach, as in cyprinids, is compensated by the supplementation of the pancreatic "trypsin" with an intestinal enzyme of the same nature. It is not clear that this hypothesis is supported by the results of Babkin and Bowie (1928) and of Ishida (1936), who also studied stomachless fish, but the suggestion is an interesting one, and the whole problem of the localization of the proteolytic enzymes of fish evidently merits further exploration.

Clarification of this problem, however, demands use of the modern concepts of proteolytic action which have so far found little space in the literature of fish digestion. The distinction now made between exopeptidases and endopeptidases has already been referred to (p. 128). The situation in the Mammalia is that the pancreas, in addition to producing trypsinogen, secretes also carboxypeptidases (possibly requiring activation), which are exopeptidases acting on those terminal bonds which involve amino acids with a free carboxyl group. The intestine contributes aminopeptidases, acting in an analogous way on amino acids with free amino groups, and dipeptidases, which complete the hydrolysis. Of these enzymes, the pancreatic products are included in the old term "trypsinogen" while the intestinal products comprise "erepsin." Vonk (1937) has emphasized that this distribution of proteolytic enzymes appears to be a characteristic not only of mammals but of all the vertebrates, the production of trypsinogen in fish being also restricted to the pancreas. His view is supported by many of the results quoted above, and is also confirmed by the work of Schlottke (1939), who has studied the distribution of proteolytic enzymes in the alimentary canal of the carp. He showed that trypsin appeared in the intestinal contents and mucosa during digestion, but that maximal amounts were found in the mucosa later than in the lumen, and he therefore concluded, in agreement with Vonk, that the mucosal enzyme had been absorbed there after being produced in the pancreas. This, it will be realized, is quite contrary to the later results of Al-Hussaini (p. 138), who did not refer to this particular work. Schlottke found polypeptidase to be more abundant in the mucosa than in the contents, and he therefore believed it to be partly intracellular, while dipeptidase, found chiefly in the mucosa and virtually absent from the contents, was held to be entirely intracellular, as also, incidentally, was the lipase. Belief in the participation of intracellular enzymes in fish digestion is in line with the findings of Wright *et al.* (1940) who, after studies on several species of mammals, concluded that the duodenal secretion contained only amylase and enterokinase and that the other enzymes associated with that region were intracellular. This is an important concept, and, quite apart from its general interest in suggesting the persistence in vertebrates of the primitive intracellular digestive mechanism, it should always be borne in mind when seeking a correlation of enzyme distribution with histological signs of the production and discharge of zymogen.

C. Carbohydrases

Investigations of the carbohydrases of fish have largely been confined to the identification of amyloclastic activity, which has been found in the pancreatic juice and extracts of *Raja* (Babkin, 1929) and in the

pancreatic extracts of other elasmobranchs (Sullivan, 1907), including *Scyllium (Scyliorhinus)* (Beauvalet, 1933a). There seems to be no record of its presence in the intestinal mucosa of these animals, but in the teleosts it has been recorded as occurring in both the pancreas and intestine, although, for the anatomical reasons already explained (p. 110), it is not always easy to separate the properties of the two organs. Bayliss (1935) found in the intestine of *Pleuronectes* a definite but not very powerful amylase with an optimum pH of 7.5 to 8, and he concluded that this was probably secreted by the intestine itself, since it was not especially associated with extracts of the caecal region which were contaminated with pancreatic tissue. He was able to show that this enzyme, like mammalian amylase, was influenced by chloride ions, the addition of 0.05 to 0.1% sodium chloride to the digest mixtures resulting in a marked increase of activity. Other records of amyloclastic activity include its presence in the intestinal mucosa of *Fundulus* (Babkin and Bowie, 1928) and of *Zoarces* (MacKay, 1929a), of the stomachless fish *Calotomus, Spheroides, Thalassoma,* and *Salarias* (Ishida, 1936), and in the cyprinids *Rutilus, Cyprinus,* and *Gobio* (Al-Hussaini, 1949b). In the last-named group the activity was most pronounced in the anterior intestinal swelling, but Ishida found that in the fish studied by him the more posterior extracts were the more potent. Such differences can have little significance, however, unless stringent precautions have been taken against the inclusion in the extracts of adsorbed pancreatic enzymes, for it seems clear that amylase certainly is produced in the pancreas in teleosts. It has, for example, been identified in that organ in *Anguilla japonica* (Oya *et al.*, 1927), in the carp (Vonk, 1927), and in *Lota* (Schlottke, quoted by Vonk, 1941), the last-named fish showing greater activity in the pancreas than in the intestine. Chesley (1934a) studied a series of teleosts in which the pancreas varied from a compact to a diffuse organ, and concluded that in the former condition it was the main center of production of enzymes, including amylase. His results indicated that when it was diffuse the pyloric caeca partly supplanted it as a source of enzymes, but it is doubtful whether his methods would have excluded the possibility of the caecal extracts being contaminated by adhering pancreatic tissue. Liver extracts may be expected to show amyloclastic activity, but there is no reason to attach digestive significance to this unless pancreatic tissue is included in the organ. This is well illustrated in *Pleuronectes* (Bayliss, 1935), in which animal, as already mentioned, the pancreas extends around the gall bladder and into the liver. Extracts of the latter organ here showed amyloclastic activity of the same order of intensity as that of the intestine, but no activity was shown by the bile unless it was allowed to extract the gall

bladder wall (and hence the investing pancreatic tissue) before removal. Al-Hussaini (1949b) found the bile of cyprinids to be "practically devoid of enzymes," except for a very slight amyloclastic activity in *Cyprinus* and *Rutilus*. It is not clear whether he attached digestive significance to this, but it should be remembered (p. 110) that it is not certain that a separate anatomical route always exists for the discharge of enzymes from the intrahepatic pancreas; the possibility that they sometimes pass through the bile duct and gall bladder cannot, therefore, be excluded.

Maltose is commonly stated to be the main product of the action of digestive amylase, and it is to be expected that maltase will be closely associated with the latter enzyme. Ishida (1936) found it to be present in the intestine of all four of the stomachless fish studied by him (see above) and in the liver of all except *Salarias*, the only genus of the four in which that organ is not penetrated by the pancreas. Vonk (1937) has emphasized that, in mammals, amylase is secreted in the pancreas (and salivary glands) with only a trace of maltase, the main production of maltase, lactase and invertase taking place in the intestine, so that there is a "chain" of carbohydrase action comparable with that of the proteolytic enzymes (see above, p. 139). While he has argued that the situation relative to the proteolytic enzymes is essentially similar in fish, he finds this not to be true for the carbohydrases, for a considerable amount of maltase activity is found in pancreatic extracts of these animals. In fact he has interpreted (1927, 1937) the very low carbohydrase activity of the intestinal mucosa of fish as indicating that the enzymes concerned are entirely adsorbed from the pancreatic secretion and are not actually produced in the mucosa at all. In the carp, the amount of maltase in the pancreas is certainly large, although its activity is only 1/25th that of the amylase, and Schlottke (quoted by Vonk, 1941) found also in *Lota* that there was more both of amylase and of maltase in the pancreas than in the intestine. It thus seems possible that the histological simplicity of the fish intestine, and particularly the absence of organized multicellular glands, may be reflected in a secretory function which is less specialized than that of mammals, at least as far as the production of carbohydrases is concerned, although the evidence does not appear to justify the view that the intestine of fish plays no part at all in that production. This latter point is emphasized by Ishida (1936) who has studied the effect of fish extracts on substrates other than starch and maltose. He found evidence for the existence of an invertase, but its site of production varied in a manner not wholly reconcilable with the views of Vonk; in *Salarias* it was present in the intestine and not in the liver, in *Spheroides* in the combined liver and pancreas but not in the

intestine, in *Thalassoma* in both organs, and in *Calotomus* mainly in the posterior intestine. A very unusual observation was the hydrolysis of amygdalin and saliein by intestinal extraets of the herbivorous *Calotomus* and *Salarias,* but not of the other two genera, both of which are earnivores. Sueh β-glucosidase aetivity is best known in the snail, *Helix pomatia,* but has also been deseribed in the mueosa of the small intestine and duodenum of the pig (see Veibel, 1950).

Cellulases and hemieellulases have been deteeted in invertebrates but have been thought to be absent from the digestive seeretions of vertebrates; Jewell and Lewis (1918), for example, found lichenase to be present in eaeh of twenty speeies of invertebrates studied by them, but absent from twelve vertebrate speeies. They emphasized, however, the need for further investigation of the lower vertebrates, and it is thus of great interest that liehenase aetivity has been reported (Ishida, 1936) in the eombined liver and pancreas and in the intestine of two teleosts (see below, p. 145), while goldfish are said to be able to digest xylan and algin (Migita and Tanikawa, 1948). In eontrast to such results, *Tilapia esculenta* (Fish, 1951, 1955), although a plankton feeder, is unable to break down the eell walls of blue-green and green algae, presumably owing to the absence of eellulase. It thus relies largely for its nutrition upon diatoms and is found to be in poor condition in water in whieh these are only present in small numbers.

D. Lipase

The proteases and earbohydrases are highly speeific for particular ehemical linkages, so that there is probably justification for the assumption, commonly made in the literature, that the hydrolysis of a partieular substrate indieates the presence of a eorresponding individual enzyme. With lipases and esterases, however, the situation is otherwise, for these are enzymes of low specifieity, with only a general requirement for an ester linkage. While different esters are likely to be hydrolyzed at very different rates by the same digest mixture, the nature of the substrate does not, in the present state of knowledge, afford any firm basis for a more preeise eharaeterization of the enzyme.

Lipase aetivity has been deteeted in the pancreatic extraets and juice of *Raja* (Babkin, 1929), and in the panereas of various elasmobranchs, ineluding *Scyllium (Scyliorhinus)* and *Squalus* (Sullivan, 1907; Vonk, 1927; Beauvalet, 1933a). In teleosts it has been found in the intestinal mueosa of *Fundulus* (Babkin and Bowie, 1928), of *Calotomus, Spheroides, Thalassoma,* and *Salarias* (Ishida, 1936), of *Zoarces* (MacKay, 1929a), in whieh animal it was weaker in the intestinal mucosa than

in the gastric (see above, p. 132), and of *Pleuronectes* (Bayliss, 1935), in which its activity was increased by the addition of bile to the mucosal extracts. Bayliss believed that the lipase of *Pleuronectes,* like the amylase, was secreted by the intestine, since lipoclastic activity was not specially associated with the pyloric cacca and their investing pancreatic tissue, and this is certainly the implication of some, at least, of the other teleostean studies quoted above. On the other hand, Chesley (1934a) found evidence for the participation of the pancreas in lipase secretion in several teleosts, and Ishida (1936) detected lipoclastic activity in the pancreas of *Salarias,* in which animal this organ is separable from the liver. Al-Hussaini (1949b) found that the distribution of lipase in the intestine of cyprinids, like that of the amylase, differed from that of "trypsin" (p. 138) in being more concentrated towards the anterior end; a contribution from the pancreas was indicated by the fact that a positive histochemical response (p. 123) for the enzyme was given by that organ as well as by the intestinal mucosa. Schlottke (1939, and quoted by Vonk, 1941) found lipase in the pancreas of the trout, but believed it to act as an intracellular enzyme in the intestine of the carp; lipoclastic activity present in the intestinal contents of this animal, and also of the perch and of *Lota,* was considered by him to be derived from the food, an aspect of digestive physiology which has received little attention by other workers. Unfortunately, the reports of the presence of lipase in extracts of the combined liver and pancreas of *Tinca* and *Cyprinus* (Beauvalet, 1933b), of *Carassius* (Sarbahi, 1951), and of *Calotomus, Thalassoma,* and *Spheroides* (Ishida, 1936) are not necessarily evidence of its production by the pancreas, since such activity might be a property of hepatic tissue.

It is evident that the source of production of this enzyme in fish is not yet clearly established; Vonk (1937) has rightly drawn attention to the way in which the results have been confused by the failure of investigators to consider the possibility of the adsorption of pancreatic enzymes on to the intestinal mucosa, and it is doubtful whether the evidence yet makes it possible to define with certainty the relative importance of the contributions of these two organs to the secretion of lipase, or, indeed, to the secretion of any other of the digestive enzymes.

IX. ADAPTATIONS OF ENZYMES TO DIET

Various authors have from time to time raised the problem of the extent to which the digestive enzymes of fish are adapted to the diet of the animals concerned. This is a question which can be asked in two forms: firstly, to what extent is the enzyme equipment of any particular

species adapted to its normal diet, and, secondly, to what extent can the enzyme equipment of a particular individual be adapted in response to changes in diet?

To the first question a positive answer can be given for the Metazoa as a whole. Yonge (1937) has stated that there is a definite correlation between the food of any animal and the nature and relative strength of its digestive enzymes, and much evidence in support of this view can certainly be adduced from the literature on fish, although Chesley (1934a) found that *Brevoortia,* consuming much fatty food, had a concentration of lipase no greater than that of other fish which consumed less fat. In the three cyprinids studied by Al-Hussaini (1949b), the concentration of carbohydrase was highest in the predominantly herbivorous *Cyprinus* and lowest in the predominantly carnivorous *Gobio,* while the situation for protease was the exact opposite. A similar relation between diet and the concentration of carbohydrases was noted by Kenyon (1925) and Vonk (1941), and the latter author concluded that the difference between omnivores and carnivores was much greater for these enzymes than for proteases. He showed, for example, that the amyloclastic activity of the pancreas of the carp was more than a thousand times greater than that of the carnivorous dogfish (*Squalus*) and pike (*Esox*), and that while there was considerable maltase activity in the pancreas of the carp and a smaller amount in the intestine, such activity was barely detectable in these organs in the pike. In contrast to these remarkable differences, the "trypsin" activity of the pancreas of the dogfish and pike was only eight times greater than that of the carp, and for the intestinal "erepsin" the difference was even less. According to Chesley (1934a), the mode of life may be of importance in such comparisons, for he found a greater concentration of enzymes in the more active types of fish.

Yonge (1937) has also drawn attention to the fact that certain animals have acquired specific enzymes which have enabled them to exploit particular types of food, striking examples being the cellulase and chitinase of *Helix.* This has not been recognized as a property of vertebrates, for, as is well known, the mammals rely upon a symbiotic flora within the alimentary canal to enable them to deal with a specialized herbivorous diet. This possibility does not appear to be open to fish, for according to Margolis (1953) it is generally held that at least as far as bacteria are concerned there are no common commensals in their intestine, the presence of a bacterial flora depending solely upon the recent intake of food and the degree of contamination of the food and water. He found that three bullheads (*Ameiurus*) and two speckled trout (*Salvelinus*) examined two hours after feeding yielded intestinal bacteria in large numbers, while of sixteen bullheads and three speckled trout examined

after a period of fasting, only one yielded a pseudomonad, and that in small numbers. Against this background the claim that certain fish possess such enzymes as amygdalase, salicinase, or lichenase (see above, p. 142) is of great interest. It is stated by Ishida (1936) that all three of these enzymes were present in the two herbivores he studied and absent from the two carnivores, although his Table 8, perhaps as the result of a misprint, indicates the reverse of this for the lichenase. As mentioned earlier (p. 113), it is most desirable that such studies of adaptations in enzyme systems should be linked with observations of the stomach contents of specimens taken under a wide range of conditions.

The answer to the second question posed above is much more doubtful. From time to time evidence for the adaptation of the enzyme equipment of individuals to particular diets has been advanced in the mammalian literature as, for example, the increase in the concentrations of amylase and maltase in human saliva in response to a carbohydrate diet and their decrease in response to protein diet (Squires, 1953). Schlottke (1939), however, in a study of the carp, found that while there were variations in the relative proportions of amyloclastic and proteolytic activity, these were not clearly correlated with the nature of the diet, although artificial food stimulated the production of a larger amount of secretion than did natural food. The latter, however, was digested more quickly, perhaps because the hydrolysis was aided by autolytic action. The response of the alimentary canal by changes in gross size to fluctuations in diet (p. 120) clearly provides a possible basis for variations in secretory activity, but the extent to which the latter actually occurs, and the degree, if any, of its specificity, are matters on which no useful opinion can at present be expressed.

X. ABSORPTION

Absorptive processes are bound to be influenced by the rate at which food passes through the alimentary canal, and this is known to be much slower in fish than in mammals. Much of the difference is doubtless associated with temperature, which has a marked effect upon the rate of activity of the canal. Thus, Maltzan (1935), by feeding one-year-old carp on colored food followed by uncolored, and noting the first appearance of the latter in the feces, showed that the time of passage of material through the alimentary canal varied from 18 hours at 10° C. to 4½ hours at 26° C. the Q_{10} ranging from 2.6 for the interval 10–20° C. to 2 for the interval 16–26° C. There are, however, other important factors, including the condition of the animal, the nature and hence the stimulatory effect of the food, and the frequency of feeding, and these will be particularly marked under laboratory conditions and may account for

the variation in the recorded data. Thus, while van Slyke and White (1911) found that a meal of chopped beef was almost completely digested by dogfish after 3 days, Weinland (1901) records the retention of food in the stomach of selachians for 3 days or more, and in one case for 18 days, and Dobreff (1927) noted defecation first occurring in a dogfish 5 days after a meal. No useful purpose would be served by comparing such results in detail, since the relevant conditions are not always fully recorded, but they do illustrate the importance of the stomach in retaining the food and delaying its passage (p. 111), and have a bearing on the problem of gastric absorption. Frequent feeding, by increasing the rate of emptying of the stomach, will tend to neutralize this delaying effect (Dawes, 1930), and this doubtless explains why Moore (1941), in experiments with twice- and thrice-daily feeding of fish, found that *Apomotis* and *Perca* did not feed continuously, but ate their fill at one or two feeding periods daily. The effect of over-frequent feeding would seem likely to be either a reduction of the amount ingested at any one time, or defecation of the material before it has been fully digested. Moore did, in fact, find that although fish would accept food more frequently than once per day, the total amount ingested, when averaged over several weeks, was no more than if they had been fed only once per day, while fish fed several times daily did not appear to gain weight more rapidly than those fed once per day. Dawes believed that there might actually be more complete absorption when food was scarce, since fish supplied with intermediate quantities of food made better use of it than those given maximum rations.

The columnar "striated border" cells of the intestine have commonly been regarded as primarily absorptive in function and there is evidence that their appearance changes in relation to periods of feeding, but it should be remembered that such changes may also be related to secretory function or to intracellular digestion (p. 139). In *Pleuronectes* (Dawes, 1929) a deep-blue, spindle-shaped mass is visible in the middle of the resting cell, in preparations stained with Delafield's hematoxylin; during digestion this is not apparent and instead a dark mass is visible just below the pale band which underlies the striated border, while the nuclei are more difficult to stain. In *Carassius* (McVay and Kaan, 1940) the columnar cells show a conspicuous granulation arranged as a band in the distal half of the cell, but in the last half of the intestine, in animals killed immediately after feeding, single vacuoles appear in this region. Rogick (1931) observed vacuoles in the cells of the intestine of *Campostoma* and regarded them as related to mucus formation, but McVay and Kaan did not support this interpretation, and the significance of the vacuoles of *Carassius* remains unexplained.

More precise attempts to localize the sites of absorption have depended upon observations on the fat content of the mucosa, and pioneer observations in this connection were carried out by Greene (1913) on the king salmon. He concluded that some absorption occurred in the stomach, although to a much less extent in the corpus than in the pyloric region, this last being a significant difference in view of the fact that the gastric glands are largely confined to the former (p. 118). The pyloric caeca in feeding fish were found to be filled with a creamy yellow viscid mass, and extremely rapid and vigorous absorption of fat was occurring there, so much so that he regarded them as the main centers of fat absorption, although it is not clear from his account that absorption was any less active in the rest of the intestine. However, other workers have described the entry of the intestinal contents into these organs. In the herring, for example, the caeca of "non-feedy" fish are pale, cream-colored, and contracted, while in "feedy" ones they are enlarged and orange to reddish in color as a result of the presence of oil and of minute particles of food. The fact that in this same fish less pepsin can be extracted from the stomach of "feedy" specimens than from "non-feedy," while the reverse is true for the extraction of trypsin from the caeca, is in agreement with the view that much digestive activity is occurring in the latter. In other fish the caeca have been variously described as possessing a highly folded and richly vascular mucosa, fine ciliation, and signs of muscle sphincters at their origin (Blake, 1930, 1936; Rahimullah, 1945), and there seems no reason to doubt that they are of importance as a site of absorption (Schlottke, quoted by Vonk, 1941).

The careful observations of Dawes (1930) on *Pleuronectes* showed that no fat was present in the mucosa of the post-pyloric regions after 6 to 7 days fasting, but that considerable quantities of fat were present in the intestinal mucosa after the digestion of a fat-containing meal. This was taken to be evidence of marked fat absorptive capacity, and the rectum differed in being capable of only some slight absorption. Like Greene, Dawes found evidence also of fat being absorbed in the stomach. Droplets were still visible in the gastric mucosa after fasting, but a marked increase had occurred after 30 and 50 hours of digestion. Since partial relaxation of the pyloric sphincter may occur at 16 to 18 hours after feeding, these time relations do not seem to exclude altogether the possibility of the transport of material to the gastric region after absorption elsewhere, but support was given to Dawes' view by the condition in animals which had received another meal soon after the first. This resulted in a great increase in the fat content of the intestinal mucosa, but only a small increase in that of the stomach, an observation which suggested that the food had passed more quickly (see above, p. 146)

and implied that the stomach might be more important in absorption when food supplies were restricted.

Al-Hussaini (1949b) fed castor oil to cyprinids which had been fasted for two to three weeks and found evidence that fat was absorbed mainly by the second and third limbs of the intestine, the anterior region absorbing less, and the "rectum" very much less. Like Dawes, he used osmium and Sudan III techniques to identify the fat droplets, and, finding that the former showed a greater concentration than did the latter, inferred that some of the fat was modified during its absorption. As mentioned earlier (p. 123), the intestinal cells are richly supplied with mitochondria, and he found that these underwent a rapid disintegration during fat absorption, losing much of their rod-like shape and becoming closely applied to the fat droplets. No such changes were seen after feeding with starch, casein, or inert material, and he inferred that these inclusions were closely involved in the mechanism of fat absorption. There is, indeed, other evidence for the involvement of mitochondria in fat metabolism, but they have also been implicated in the production of both extracellular and intracellular enzymes (Bourne, 1951), and their function in these particular cells clearly merits further study.

The work of van Slyke and White (1911) stands rather alone in the literature as a considerable attempt to study by biochemical means the digestion and absorption of protein in fish. They fed known amounts of chopped and boiled beef to dogfish and found that after 6 hours a considerable part of the protein in the stomach was dissolved, the average size of the peptides being at that time pentapeptides. Although the passage of stomach contents into the intestine had hardly yet begun, at least 25% of the ingested protein could not be accounted for, and they concluded that absorption must have taken place from the stomach. Later, the material passed into the intestine where further absorption would take place, and, as already mentioned (p. 146), digestion was almost completed after 3 days; at no time, however, did protein breakdown proceed further than the dipeptide stage, either in the stomach or intestine, a particularly interesting observation in view of present-day suggestions as to the completion of proteolysis by intracellular enzymes (p. 139). Of interest also, in the light of the cytological evidence for gastric absorption of fat, is their observation that the digestion of protein in the stomach freed the accompanying fat, which was readily detectable in the stomach contents, although it could not be found in the contents of the intestine. They interpreted this as indicative of rapid lipolysis in the intestine but they do not appear to have considered the

possibility of some gastric absorption. This, however, seems likely since fat absorption is reported to take place in *Cetorhinus* in the bursa entiana, which is a specialization of the pyloric region of the stomach (Matthews and Parker, 1950).

Evidence for the expected occurrence of absorption in the intestine of fish seems clear enough from the above results, although much remains to be learned regarding the details of the process. Indications of absorption taking place in the stomach may seem more surprising, but such evidence is not, in fact, confined to fish alone. Karel (1948), in a review of gastric absorption, considered mainly from the standpoint of mammalian physiology, has concluded that its occurrence cannot be lightly discounted, although the fact that histological evidence of fat absorption in the stomach has not been confirmed by chemical analyses of the blood may indicate that the amount so absorbed is very small. It seems clear that positive evidence for gastric absorption in fish is prominent in the rather few studies dealing with this field, and the composition of the gastric secretion of fish merits further study from this point of view, for, as has been seen above (p. 131), the relevant data are at present inconclusive. It is certainly unsatisfactory that cytological evidence for the gastric absorption of fat in *Pleuronectes* could not be correlated with the presence of gastric lipase (Bayliss, 1935), but Schlottke's conclusions relative to the intracellular action of intestinal lipase and to the lipoclastic activity of the food itself (p. 143) may be of significance here, nor can the possibility of regurgitation of intestinal fluids be neglected (p. 131). The problem is one which also merits much more attention from the cytological standpoint. The striated border, for example, with its associated alkaline phosphatase (which has been shown by Al-Hussaini, 1949b, to occur in the border of the intestinal cells of fish), has been thought to be implicated in absorptive processes, but no such border occurs in the stomach. Again, the absorption of the products of protein digestion would seem to be bound up with the existence of intracellular dipeptidases, and these also require investigation. The problem presented by fat absorption is possibly simpler, for, apart from the considerations mentioned above, there is some reason for believing that this does not in any case demand complete lipolysis, and that fat can pass into the mucosa as minute fat droplets. Although droplets have been observed in the striated border of mammalian intestinal cells (Baker, 1951), it is not even clear that the presence of this structure is essential for their transit, for it has been asserted that they can also pass between the individual cells (Hewitt, 1954), a possibility which again merits investigation in fish.

XI. THE CONTROL OF DIGESTIVE FUNCTIONS

A. Movement

The motor and secretory activities of the alimentary canal of mammals are under the influence of a complex system of nervous and endocrine controls. This aspect of digestive physiology has, unfortunately, been as yet but little studied in fish, but the results which have been obtained certainly suggest that while in principle the situation may be the same, there are many differences in detail which in some instances are clearly referable to the lower evolutionary status of the group (p. 111).

In elasmobranchs, as in mammals, the alimentary tract is innervated by vagal and sympathetic fibers, but there is reason to believe that the distribution of the two components does not overlap as extensively as in the latter group. In selachians the esophagus and corpus are innervated by the vagus, which extends back on the right side on to the pyloric region. Young (1933) could not trace any vagal branches posterior to the pylorus in *Scyliorhinus,* while Babkin *et al.* (1935b), investigating *Raja,* found the vagus disappearing within the muscular tissue at about the middle of the corpus, and were unable to follow it further "with the naked eye." Müller and Liljestrand (1918), however, have stated that a branch of the right vagus extends on to the spiral intestine in *Raja,* and Müller (1920) found the vagus reaching the intestine in developing *Squalus,* while in the stomachless chimaeroid *Hydrolagus* (Nicol, 1950), the autonomic system of which closely resembles that of selachians, the vagus runs to the esophagus and intestine. There is in these fish no sacral parasympathetic system; this seems to appear first in terrestrial vertebrates in connection with the development of the cloacal bladder. An extensive sympathetic supply, however, runs the whole length of the alimentary tract, from esophagus to rectum, arising from the paired sympathetic ganglia and passing directly to the gut as nonmedullated fibers in the anterior, median, and posterior splanchnic nerves.

Young (1933) found that faradic stimulation of the vagus in selachians regularly caused contractions of the esophagus and often also of the corpus, but no effects, either motor or inhibitory, were observed posterior to this region, so that there was no physiological evidence of vagal innervation of the intestine. Stimulation of the sympathetic through the anterior splanchnic nerves or associated regions produced contraction in the stomach, while stimulation of the middle or posterior splanchnic nerves, or associated regions, produced variable but undoubted contraction of the intestine or rectum. It seems clear from his work, therefore, that both vagal and sympathetic fibers are motor to the alimentary musculature, and essentially similar results were obtained in *Squalus* by

Lutz (1931) and in rays by Babkin *et al.* (1935b), who also observed that sympathetic stimulation produeed even stronger contraetion of the stomaeh than did vagal, and that both together showed a summation effeet. Neither these workers nor Young found any indieation of an inhibitory effeet of the vagus upon the intestine, sueh as had been reported by Müller and Liljestrand, and Young points out that the results of the latter, who also obtained no response of the intestine to stimulation of the middle and posterior splanehnie nerves, may have been confused by their moistening of the viseera with sea water, which laeks the urea present as a normal constituent of the body fluids of elasmobranchs.

Experiments with autonomie drugs have given essentially similar results. Aeetyleholine has been found to stimulate isolated pieces of the stomach (Nicholls, 1934), as also does adrenaline (Lutz, 1931; Young, 1933), while the latter drug, when injeeted into the lateral vein of rays, produees movements of the stomaeh identieal with those resulting from stimulation of the anterior splanehnie nerves (Babkin *et al.*, 1935b). There are, inevitably in sueh diffieult studies, some eontradictions. Young, for example, found that adrenaline stimulated the rectum while Lutz found it to be inhibitory. Again, atropine has been reported to stimulate gastrie eontraetions and actually to increase the effeet of vagal stimulation (Babkin *et al.*, 1935b), while it abolished the stimulating effeet of acetylcholine on strips of gastric musele. It is elear, however, that the results, both physiologieal and pharmaeologieal, are in broad agreement in showing the absenee from selaehians of that funetional antagonism between the two autonomie eomponents which is a familiar feature of mammalian organization.

The teleosts have been less extensively studied from this point of view, but it is known, mainly from the work of Young (1931, 1936), on *Uranoscopus* and *Lophius*, that all parts of the stomaeh and intestine are innervated by the sympathetie system through the large anterior splanchnic nerves, and that the vagus supplies the esophagus and stomaeh; an extension of the latter to the intestine has not been clearly demonstrated, either anatomieally or physiologieally, so that here, as in selaehians, there is only a limited overlap in the distribution of the two components. Young found, by faradie stimulation of the nerves *in situ*, that the vagus was motor to the stomach and the splanehnie nerves motor to the intestine, but there was no evidenee for a vagal effect upon the intestine, and only slight indieations of sympathetie stimulation of the stomaeh. Investigation of the effeets of drugs upon isolated strips of the alimentary canal of these fish showed that aeetylcholine increased the tonus of all parts while adrenaline deereased it,

so that there was no agreement between the effect of the drugs and those of their corresponding autonomic components. Adrenaline and pilocarpine, it should be added, have no effect at all upon the esophagus of teleosts which, it will be recalled (p. 110), is supplied with striated muscle (Nicol, 1952).

B. Secretion

Gastric secretion in mammals is controlled through a combination of nervous and hormonal paths (Babkin, 1950), but little is known as to the existence of comparable mechanisms in fish, and only the selachians have been seriously studied from this point of view. The existence in the latter of a causal relation between secretion and the ingestion of food is indicated by the fact (p. 126) that after feeding there is a marked increase in the volume and acidity of the gastric juice, the acidity reaching a maximum about one or two days before the emptying of the stomach, and thereafter slowly falling. Ungar (1935), who was able to collect a small amount of secretion from perfused isolated stomachs of *Squalus, Scyliorhinus,* and *Torpedo,* found some evidence that histamine and acetylcholine stimulated secretory activity, and that atropine inhibited the effect of the latter drug, but investigations carried out on entire animals have unfortunately so far failed to establish the existence of any mechanism influencing the gastric secretion of selachians. Dobreff allowed several dogfish to attempt to secure prey while preventing them from actually swallowing it, and, after twenty minutes of these efforts, removed samples of their gastric contents with a glass siphon. He found no change in the fasting gastric secretion, and concluded that the "psychic" or "appetite" phase of secretion, mediated in mammals through the vagus and stimulated by the sight or smell of food, did not exist in these fish. Intramuscular injections of pilocarpine, acetylcholine, and atropine were also without effect, and he was, in short, unable to find any evidence at all for nervous control. Similar results were obtained by Babkin *et al.* (1935a) in their studies of *Raja,* which involved not only intact animals but also others which were kept alive under artificial respiration after anesthesia or cutting of the spinal cord. Induction of swallowing movements by the insertion of a balloon into the esophagus, section of the vagi, distention of the stomach by a balloon or by food, stimulation of the anterior splanchnic nerves, and injection of adrenaline or atropine were all without effect, a result which, as they pointed out, was the more remarkable in view of the fact that motor innervation of the stomach is well developed (p. 150) and was clearly demonstrated during these actual experiments.

Neither these authors nor Dobreff were able to demonstrate any effect

of injections of histamine upon gastric secretion. This substance is well known to be a powerful stimulant of acid secretion in mammals, and it has, in fact, sometimes been supposed that it may be a naturally acting humoral agent (Babkin, 1950). The situation here, however, is complicated by the fact that the response of selachians to histamine is strikingly different from that of warm-blooded forms (MacKay, 1931); intravenous doses of as much as 100 mg. are without effect upon rays, and the substance appears to be relatively harmless to them, producing at most a slight fall in blood pressure which is very insignificant compared with that produced in mammals.

It must be concluded, then, that there has as yet been no convincing demonstration of either nervous or humoral control of gastric digestion in selachians, but it is hardly possible to remain satisfied with such a negative position. It should be remembered that there is little information as to the preeautions which must be taken if these fish are to remain in a satisfactory physiological condition during prolonged observation and experimentation, and the negative results may merely reflect a failure to aehieve such a condition. The results of Ungar, quoted above, should be some encouragement to those who wish to pursue further these admittedly difficult investigations.

Pancreatic secretion in mammals, like gastric secretion, is under nervous and hormonal control. As regards the former, the sympathetic system possibly exerts its effect entirely through vasomotor responses (Riehins, 1953), but the vagus stimulates the discharge of zymogen. The hormones involved are secretin and pancreozymin, the former stimulating the discharge of fluid and the latter the discharge of zymogen; both of these may be present in crude preparations of "seeretin," in the earlier usage of this term (Harper and Raper, 1953).

Babkin (1929, 1933), using rays which were immobilized either by anesthesia or by seetion of the spinal cord, was able to demonstrate the spontaneous production of a very small quantity of pancreatic secretion, and showed that this was almost certainly capable of being influenced by a humoral path. The amount of secretion could be increased by the introduction of a solution of hydrochloric acid into the intestine, and this effect was augmented by the previous introduction of an acidified peptone solution or of an acid digest of fish tissue. Increase of secretion also followed the injection into the gastric veins of a "secretin" preparation, and, since Bayliss and Starling (1903) had previously demonstrated the existence of this hormone in the intestine of the dogfish, skate, and salmon, it seems likely that this particular hormonal mechanism can be regarded as a fundamental property of the alimentary tract from fish to mammal. On the other hand, injections into the blood stream of

pilocarpine, acetylcholine, choline, atropine, adrenaline, and histamine had no effect at all upon the volume of secretion, apart from effects which were evidently vasomotor in origin, and Babkin therefore concluded that there was neither parasympathetic nor sympathetic control of pancreatic secretion in the ray.

In mammals there is evidence that "secretin" promotes the secretion of bile, and that another hormone, cholecystokinin, stimulates contraction of the gall bladder. Some indications that a similar mechanism may be operative in fish are provided by Babkin's observation that introduction of hydrochloric acid and of peptone solution into the intestine of rays increased and sometimes doubled the rate of bile secretion. Babkin and Bowie (1928), studying the stomachless teleost *Fundulus*, noted that twelve hours after a meal the intestine was filled with food and bile, and that the gall bladder was collapsed and empty, while in fish that had not fed the latter was still fully distended. Mechanical stimulation of the intestine, promoted by feeding the animal with cork and rubber, produced no discharge of bile, whereas introduction into the intestine of a few drops of 1% pilocarpine provoked motor phenomena, including defecation and the discharge of bile. These observations, made incidentally during the course of work directed to other ends, are too fragmentary to permit of decisive interpretation, but they are indicative of the type of problem presented by this aspect of the physiology of digestion in fish.

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Chapter IV

EXCRETION AND OSMOREGULATION

Virginia Safford Black

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I. INTRODUCTION

The functions to be discussed in this chapter are of particular interest from the point of view of animal evolution. The study, in time and space, of the nature of body fluids and control of their osmotic concentration in animals is a fascinating one (Pantin, 1931). Dakin (1935) has pre-

sented an excellent discussion of the subject for aquatic animals, and Smith (1932) for fishes only.

Paleontological and anatomical evidence indicates that fishes first evolved in fresh water, and gradually invaded the sea (Romer and Grove, 1935). The kidney structure of fishes favors osmotic regulation in a freshwater medium, and this fact supports the theory of the freshwater origin of vertebrates. Since the quantity and variety of salts in both fresh and sea waters is different from that in the body fluids of fish, all fish (except the myxinoids, see Section II, B, 3, a) must perform the function of osmotic regulation, i.e. fishes are homeosmotic. The physiological adjustments which are made in performing this function are truly remarkable.

The functions of excretion and osmoregulation are usually closely related in animals and are performed by the same structures. In fish these structures are the gills and the kidneys. The gills are filamentous tissues supported by a gill arch and protected by the body wall or an operculum. The blood supply consists of afferent arteries, from the ventral aorta, which divide into capillaries in the gill filaments and come together again to form the efferent arteries leading to the dorsal aorta. Although the gills function chiefly as a respiratory organ (see Chap. I: Part I), they do very important work in excretion and osmotic regulation.

The comparative anatomy of the kidney has been ably presented by Marshall (1934). The typical vertebrate kidney structural unit (kidney tubule) consists of the renal corpuscle (vascular glomerulus in Bowman's capsule), a neck segment connecting with the proximal convoluted segment, and a distal convoluted segment leading into the initial collecting tubule. In fishes these parts are not always typical and one or two may be lacking altogether. Many such tubules make up the kidneys which, in fish, are elongated paired structures lying in the dorsal wall of the body cavity. The tubules of each kidney empty into a ureter which opens into the urinary bladder (urogenital sinus in cyclostomes, cloaca in elasmobranchs). The urine is then voided through the urogenital opening in the body wall. The blood supply to the kidney tubule in vertebrates is shown in Fig. 1 (Smith, 1953). Figure 1B represents a typical glomerular fish kidney tubule. The change in blood flow around the tubule in mammals with no renal portal vein is well shown. Glomeruli in all vertebrate classes receive arterial blood from the dorsal aorta, which acts as a pump assisting the passage of water and other substances from the blood to the kidney tubule. It is this efficient mechanism for facilitating the removal of water that indicates the freshwater origin of vertebrates. The tubules receive blood from the efferent glomerular artery and the renal portal vein (not present in cyclostomes

or mammals). All blood leaving the kidney in fish goes to the post-cardinal veins (post-caval vein in amphibians, birds, and mammals).

The description of excretion and osmotic regulation in fish which follows is necessarily condensed, but full accounts are available in reviews by Smith (1932, 1936), Marshall (1934), Krogh's book, "Osmotic Regulation in Aquatic Animals" (1939), reviews by Fontaine and Koch (1950) and Black (1951), and in a recent monograph by Wikgren (1953). The comparative aspects are ably presented in books by Baldwin (1948), Davson (1951), and Smith (1953). A review table of the osmotic con-

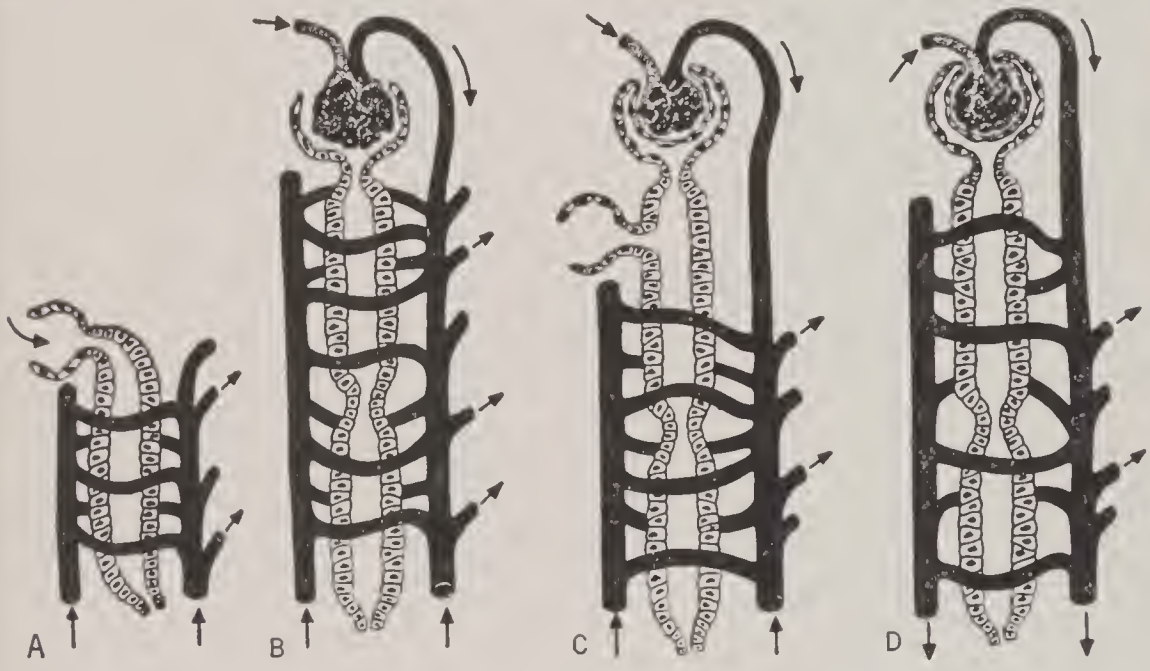

FIG. 1. Four stages in the evolution of the vertebrate nephron. A: protovertebrate; B and C: early vertebrates; D: mammal ("renal portal system" has disappeared, leaving tubules supplied only by postglomerular blood) (from Smith, 1953).

centration of blood, urine and/or tissues of more than 100 species of fish is available from the author, and will eventually be published, in part, in the section of plant and animal physiology of the "Handbook of Biological Data" now being compiled by the National Research Council of the United States. Selected data appear in Table I.

The author has received many helpful comments on the manuscript from Dr. Homer W. Smith, Dr. W. S. Hoar, Dr. Roy P. Forster, Dr. Wilbur Sawyer, and Dr. D. K. Meyer; however, full responsibility for errors, omissions, and interpretations rests with the author.

II. STENOHALINE FISHES

The following section deals with the basic mechanisms for excretion and osmoregulation in fishes that can tolerate only a narrow range of salt concentration in the water (stenohaline). In studying osmotic regula-

TABLE I

OSMOTIC CONCENTRATION MEASURED BY THE FREEZING POINT DEPRESSION OF THE BODY FLUIDS AND TISSUES OF FISHES

Species	External Environment (Δ ° C.)	Blood (Δ ° C.)	Urine (Δ ° C.)	Tissues (Δ ° C.)	Remarks	Reference
CYCLOSTOMES						
1. *Lampetra* (= *Petromyzon*) *fluviatilis*						
	Fresh water	−0.46				Galloway (1933)
	−0.57	−0.52			After 22 hrs.	Galloway (1933)
	−0.86	−0.63			After 22 hrs.	Galloway (1933)
2. *Petromyzon marinus*						
	−0.01	−0.51	−0.032			Sawyer (1955)
	−2.3	−0.59				Burian (1910)
3. *Myxine glutinosa*						
	−1.88	−1.92				Smith (1932)
ELASMOBRANCHS						
4. *Mustelus canis*						
	−1.82	−1.86 to −1.90				Garrey (1905)
	−0.90	−1.60			After 3 hrs.	Garrey (1905)
	Fresh water	−1.55			After 1 hr.	Scott (1908)
5. *Squalus acanthias*						
	−1.85	−1.95	−1.87			Smith (1931)
	−1.33	−1.62				Smith (1931)
6. *Pristis microdon*						
	Fresh water	−1.02	−0.10			Smith and Smith (1931)
7. *Raia* sp.						
	−1.48	−1.61	−0.90			Smith (1931)
	−1.09	−1.37	−0.54			Smith (1931)
CHONDROSTEAN						
8. *Acipenser sturio*						
	−2.0	−0.76				Rodier (1899)

HOLOSTEAN					
9. *Amia calva*					
	−0.03	−0.54	−0.07		Smith (1932)
TELEOSTS					
10. *Salmo salar*					
	Fresh water	−0.64			Benditt *et al.* (1941)
	−0.87	−0.77			Benditt *et al.* (1941)
11. *Salmo irideus* (= *gairdnerii*)					
	−0.02	−0.50	−0.8		Busnel (1942)
12. *Oncorhynchus tschawytscha*					
	−1.92	−0.76			Greene (1904)
	−0.02	−0.74			Greene (1904)
	−0.02	−0.67		Spawning ♂	Greene (1904)
	−0.02	−0.61		Spawning ♀	Greene (1904)
13. *Cyprinus carpio*					
	−0.02	−0.49	−0.07 (Martret, 1939)		Portier and Duval (1922a)
	Fresh water	−0.53	−0.66 to −0.70		Leövey (1938)
	−0.70	−0.80	−0.94		Leövey (1938)
	−0.71	−0.70	−0.67		Martret (1939)
	−0.90	−0.89	−0.85		Martret (1939)
14. *Carassius carassius*					
	−0.02		−0.67		Pora (1939)
	−0.90		−1.12 (not acclimated)		Pora (1939)
	−0.90		−0.97 (acclimated)		Pora (1939)
15. *Rutilus rutilus*					
	Fresh water		−0.51 to − 0.54		Gueylard (1924)
	−0.65		−0.63	After 2 days	Gueylard (1924)
16. *Anguilla rostrata*					
	−1.85	−0.82	−0.79		Smith (1932)
	−0.08	−0.63	−0.09		Smith (1932)
17. *Anguilla anguilla*					
	Sea water	−0.78			Portier and Duval (1922b)
	Fresh water	−0.63			Portier and Duval (1922b)

TABLE I (Continued)

OSMOTIC CONCENTRATION MEASURED BY THE FREEZING POINT DEPRESSION OF THE BODY FLUIDS AND TISSUES OF FISHES

Species	External Environment (Δ ° C.)	Blood (Δ ° C.)	Urine (Δ ° C.)	Tissues (Δ ° C.)	Remarks	Reference
18. *Conger conger*						
	−2.30	−1.03	−0.82			Burian (1910)
	−1.70	−0.70 to −0.79				Dekhuyzen (1905)
	−1.0	−0.80			After 8 days	Boucher-Firly (1935)
19. *Gadus virens*						
	−1.73	−0.76	−0.63			Dekhuyzen (1905)
20. *Perca fluviatilis*						
	Fresh water			−0.65 to −0.68		Gueylard (1924)
	Fresh water			−0.61 to −0.66		Leövey (1938)
21. *Tautoga onitis*						
	−1.80	−0.70 to −0.88		−0.75 to −0.78		Leövey (1938)
	−2.85			−0.8	After 3 days	Leövey (1938)
				−1.19	Lethal tissue concentration	Leövey (1938)
22. *Gobio gobio*						
	Fresh water			−0.55		Gueylard (1924)
	−0.65			−0.63	After 2 days	Gueylard (1924)
23. *Scorpaena porcus*						
	−2.16		−0.82			Martret, 1939
	−0.64		−0.64			Martret, 1939
	−0.54		−0.62			Martret, 1939
24. *Scorpaena scrofa*						
	−2.30	−0.71	−0.65			Martret, 1939
25. *Myoxocephalus octodecimspinosus*						
	−1.85	−0.82	−0.78			Smith (1932)
	Sea water	−0.72	−0.62			Forster (1953)

25a. *Myoxocephalus scorpius*					
Sea water	−0.71	−0.58 to −0.62			Forster (1953)
26. *Gasterosteus aculeatus*					
Fresh water			−0.74 to −0.76		Gueylard (1924)
−1.25			−0.74	After 1 day	Gueylard (1924)
−1.25			−0.92	After 1 day, during spawning	Gueylard (1924)
Fresh water	−0.60				Koch and Heuts (1943)
−0.60	−0.62				Koch and Heuts (1943)
−1.90	−0.65				Koch and Heuts (1943)
27. *Pleuronectes flesus*					
−1.66	−0.96				Dakin (1908)
−0.95	−0.58	−0.18			Henschel (1936)
−0.3	−0.52	−0.07			Henschel (1936)
28. *Platichthys* (= *Pleuronectes*) *platessa*					
−1.70	−0.67				Dekhuyzen (1905)
−0.95	−0.65	−0.31			Henschel (1936)
−0.3	−0.38	−0.10			Henschel (1936)
29. *Lophius piscatorius*					
−2.3	−0.77 to −1.04	−0.64 to −0.78			Burian (1910)
Sea water		−0.68			Grollman (1929)
Sea water	−0.77	−0.75			Brull and Cuypers (1954a)
Sea water	−0.67	−0.57			Forster (1953)
DIPNOI					
30. *Protopterus aethiopicus*					
−0.03	−0.48	−0.09			Smith (1930b, 1932)

tion several investigators (Grafflin, 1931; Clarke, 1934; Meyer, 1948; Brull and Nizet, 1953; Forster, 1953; Wikgren, 1953) have found that a "laboratory diuresis" occurs in some species. After the fish are caught and put in aquaria they lose salts and water at an abnormally high rate. This behavior may continue until death (often true of marine teleosts), or the fish may recover after 10 to 48 hours (freshwater fishes). The cause is not known, although injury and shock in handling have been suggested. Further discussion of this phenomenon appears in Section II, B, 3, d. Investigators should be aware of this reaction, however, not only when doing their own experiments, but also when evaluating the literature.

A. Freshwater Fishes

1. Nitrogenous Excretion (Table II)

a. Cyclostomes. Lampreys may be potamodromous or anadromous and their kidney structure is similar to that of the freshwater teleosts (Section II, A, 1, c) but more primitive. In the adult the kidney is a mesonephros (opisthonephros); in the larval stages the pronephros may still be functional. The pronephric duct becomes the "ureter" in the adult. Literature concerned with nitrogenous excretion in these fish appears to be scarce. Probably the description of excretion in freshwater teleosts would apply also to lampreys.

b. Elasmobranchs. Freshwater elasmobranchs are discussed with marine elasmobranchs (Section II, B, 1, b) because they have reinvaded fresh water from the sea and have experienced no fundamental changes in nitrogenous metabolism as a result. Elasmobranchs are essentially marine and have inhabited the sea for a longer geologic period than the teleosts (Romer and Grove, 1935).

c. Teleosts and other Actinopterygians. The typical kidney tubule of the freshwater teleosts consists of glomerulus, open neck segment, proximal convoluted tubule which is divided into two cytologically different segments, distal convoluted tubule, and initial collecting tubule. Urine is formed by glomerular filtration into the kidney tubules. Some nitrogenous wastes are excreted from the gill surfaces in the form of ammonia and urea (Smith, 1929). At the same time Delaunay (1929) also presented evidence for extrarenal excretion of nitrogen. Smith found that in the carp, *Cyprinus*, and goldfish, *Carassius*, six to ten times as much nitrogen is excreted by the gills as by the kidneys. In general, he concluded that the gills excrete ammonia and urea; the kidney excretes creatine, creatinine, and uric acid.

d. Lungfishes (Dipnoi). The structure of the kidney tubule of the

lungfish (*Lepidosiren paradoxa*) is typical of freshwater fishes (Guyton, 1935), although Grafflin (1937a) found that the second part of the proximal tubule is not homologous with other species, and the first part is homologous to the second part in freshwater teleosts.

One interesting feature of this group is the ability to estivate with a complete cessation of urine excretion. A marked uremia develops during this period, but the urea and some sulfate are rapidly excreted soon after the lungfish (*Protopterus aethiopicus*) is returned to water. Creatine, creatinine, ketone bodies, uric acid, and ammonia (toxic) are not accumulated during estivation (Smith, 1930b). According to Smith the fact that ammonia does not accumulate indicates that ammonia is normally formed in fishes not from the metabolism of protein, but peripherally from some nonprotein precursor.

2. Water Balance

Freshwater fishes are continually faced with the need to dispose of the water which they absorb osmotically because the concentration of their body fluids is greater than that of their environment. The freezing point depression of the blood of freshwater cyclostomes is about —0.46° C.; of freshwater elasmobranchs, about —1° C.; of freshwater teleosts, about —0.57° C.; and of fresh water, near 0° C. (see Fig. 4, p. 187). Most of the water is taken in through the semi-permeable gill and oral tissues, although some also crosses the less permeable body surface. The permeability of the body surfaces is specific; the time required for 1 ml. water to pass across 1 sq. cm. of membrane at a pressure difference of 1 atmosphere is 91 days for the lamprey (*Lampetra fluviatilis*) (Wikgren, 1953); 158 days for the goldfish (Krogh, 1939); and about 5 years for the eel (Keys, 1933). The only other water intake in freshwater fishes is that accompanying or bound in the food swallowed.

All freshwater fish (except the pipefish, *Microphis boaja*) have glomerular kidneys. The water taken in osmotically and formed metabolically is carried by the blood and is filtered through the glomeruli of the kidneys, because of the pressure difference between blood and filtrate. The water passes through the kidney tubules and is finally excreted in the hypotonic urine. Haywood and Clapp (1942) found an average freezing point depression of —0.025° C. for the urine of the catfish (*Ameiurus nebulosus*); and —0.094° C. for the common sucker (*Catostomus commersonii*). Values for other freshwater fishes are listed in Table I, and indicate the very dilute nature of urine in freshwater fishes.

Measurements of urine flow in some freshwater fishes are given in Table III. The amounts depend on the area and permeability to water

TABLE II

NITROGEN CONSTITUENTS OF THE URINE OF FISHES EXPRESSED IN MG. %

Species	Total N	Urea N	NH_3	Uric acid	Amino acid	Creatinine	Creatine		Rest (%)	Reference
FRESHWATER FISHES										
Elasmobranch										
Pristis microdon	66	40	4.5				3.3		28	Smith and Smith (1931)
Holostean										
Lepisosteus osseus	12.1	0.6	4.1	0	0.5	0.1	0.4		53	Smith (1929)
Teleosts										
Anguilla	77	8.2	9.0	1.0		2.0	14.8		56	Smith (1929)
Anguilla	11	2.6	2.6	0.6		0.1	3.3		17	Smith (1929)
Cyprinus carpio	49.1	Trace	10.6	0.3		1.4	16.5		41	Smith (1929)
Cyprinus carpio	27.2	1.0	Trace	Trace		0	8.6		62	Smith (1929)
Ictalurus furcatus	21.5	5.3	5.9	0	4.3	0.6	1.1		17	Smith (1929)
Carassius auratus	51.6	Trace	3.2	Trace		0	9.9		75	Smith (1929)
Carassius auratus	28.6	Trace	4.5	0.3		0	3.4		71	Smith (1929)
MARINE FISHES										
Elasmobranchs										
Raia stabuliforis	145	74					83			Smith (1931)
Raia sp. in Δ −1.09° C.	521	167					104		50	Smith (1931)
Squalus acanthias	391	344							12	Smith (1931)
Squalus acanthias	123	100							19	Smith (1931)
Torpedo	396	336	6.7		6.7				12	Delaunay (1929)
Teleosts							Trimethylamine oxide			
Lophius piscatorius	75	21	1.5		3.8				65	Delaunay (1929)
Atlantic	373[a]	0.7	1.3	0.4	14.3	4.1	27.9	+	<89	Grollman (1929)
Atlantic	337[a]	0.6	1.0	0.2	18.0	8.0	156	+	<45	Grollman (1929)
Aquarium	56[a]	2.7	0.7	Trace	4.5	0.9	12.9	+	<61	Grollman (1929)
Soon after capture	346[a]	5.3	2.1						98	Brull and Nizet (1953)

Soon after capture	54[a]	2.3	2.2						92	Brull and Nizet (1953)
Pooled	148.4[a]	1.6	19.2	0.03	4.5	4.3	51.2	28.4	26	Brull and Nizet (1953)
Pooled	100%	5.7%	56%	0.2%	0%	6.5%		28.2%	3	Baldwin (1948)
Pooled, preserved	58.8[a]		19.2	(Trimethylamine, 3)		1.4	19.9	14.3	2	Brull and Cuypers (1954a)
Opsanus tau	13–132	2.1–8.4								Marshall (1930)
Muraena [b]	102[a]	41.7	23.2		14.4	11.0	5.4			Edwards and Condorelli (1928)

[a] Nonprotein nitrogen.

[b] Representative values, not from same sample.

TABLE III

MEASUREMENTS OF URINE FLOW AND CHLORIDE LOSS IN FISHES

Species	Urine Flow (ml./kg./day)	Urine Cl. (m$M/l.$)	Diffusion Cl Loss (μM/100 g./hr.)	Reference
FRESHWATER FISHES [a]				
Cyclostomes				
Lampetra fluviatilis	362	0.7	1.0	Wikgren (1953)
Petromyzon marinus	159	4.7		Sawyer (1955)
Elasmobranch				
Pristis microdon	250	5.5	5.8	Smith and Smith (1931)
Holostean				
Lepisosteus osseus	19.2			Smith (1929)
Teleosts				
Salmo irideus	60–106	1.9–11.8	0.5–5.1	Krogh (1937)
Cyprinus carpio	48			Smith (1929)
Carassius auratus	40–100	2.5–4.1	0.4–1.7	Krogh (1937)
Carassius auratus	52.8			Smith (1929)
Catostomus commersonii	7–26	27.6 (total salt)	(0.3–1.0)	Haywood and Clapp (1942)
Ameiurus nebulosus	51–79	7.3 (total salt)	(0.5–0.8)	Haywood and Clapp (1942)
Anguilla	15, 35			Smith (1929)
Platichthys flesus (in 4 ‰ salinity)	24	19.6 (Cl)	1.7	Henschel (1936)
MARINE FISHES				
Elasmobranchs [b]				
Scyliorhinus catulus	4.2			Baglioni (1906)
Scyliorhinus catulus	2–4			Burian (1908–09)
Scyliorhinus stellare	5.3			Baglioni (1906)
Mustelus canis	18			Denis (1913)
Mustelus canis	21.6	262		Scott (1913)

Mustelus canis	15.8		Kempton (1953)
Squalus acanthias	18		Hoskins and Hoskins (1918)
Squalus acauthias	4.7–12		Marshall (1931)
Squalus acanthias	12–24	173+	Clarke and Smith (1932)
Squalus suckleyi	33.6		White (1931)
Teleosts			
Opsanus tau	0.6–9.4	0+	Marshall (1930)
Opsanus tau	< 3.6	86+	Grafflin (1931)
Lophius piscatorius	20–30		Burian (1908–09)
Lophius piscatorius	18		Edwards and Condorelli (1928)
Lophius piscatorius	12.9–54.4	72+	Marshall (1930)
Lophius piscatorius	<29.4	13	Forster (1953)
Myoxocephalus octodecimspinosus	3.2+		Marshall (1930)
Myoxocephalus octodecimspinosus	< 4	0	Grafflin (1931)
Myoxocephalus octodecimspinosus	3	0–29	Clarke (1934)
Myoxocephalus octodecimspinosus	9.3	21	Forster (1953)
Myoxocephalus scorpius	2	9	Forster (1953)
Cryptacauthodes	1.5–11		Marshall (1930)
Scorpaena	10–12		Burian (1908–09)
Conger	3–5		Burian (1908–09)
Muraena helena	5		Edwards and Condorelli (1928)
Anguilla	0.4–5	76+	Smith (1930a)

[a] Data mostly from Table 3, Wikgren (1935).
[b] Data mostly from Smith (1931).

of the body surface; conversely, a rough estimate of permeability may be derived from the amounts of urine excreted.

Although extracts of the fish pituitary gland exert an anti-diuretic effect on mammals and cause water retention in frogs, no anti-diuretic effect of this hormone has been observed in fish, even when there is a need for water conservation (Fontaine and Raffy, 1950 and see Chap. VI). In freshwater fishes, of course, conservation of water would be a hazard, but in marine teleosts water is a valuable commodity.

A discussion of water balance in the lamprey appears in Section III.

3. Salt Balance

Since the osmotic concentration of the blood of freshwater fishes is maintained at a higher level than that of the water, some salts are lost by diffusion through surface tissues. Salts are also lost in the feces and urine. In all freshwater fishes (cyclostomes, elasmobranchs, teleosts), however, the loss of salt is kept at a minimum by reabsorption in the proximal convoluted tubules of the kidneys.

Salts are replaced either by salts contained in ingested food, or by the active absorption of salt ions from the surrounding water by the gills. The latter has been studied in teleosts and in the lamprey, *Lampetra fluviatilis* (Wikgren, 1953) (and further discussion of salt balance in the lamprey appears in Section III). Krogh (1939) has interpreted Smith's data on the freshwater elasmobranch, *Pristis,* to indicate that chloride absorption may occur.

Since many species of fish undergo long periods of fasting during spawning and migration periods, the ability of the gills to take up salt ions from the surrounding water against the diffusion gradient is of prime importance. Krogh first demonstrated the active absorption of ions in 1937 for seven species of freshwater teleosts, and he has reviewed this work in his book (1939). Krogh found species differences in the ability to absorb chloride and in the threshold of chloride concentration of the water from which the ion could be taken. Wikgren (1953) has compiled tables showing thresholds and rates of chloride absorption in some species of aquatic animals. The values given for fishes are reproduced in Tables IV and V.

Krogh noted lack of absorption, often for several days, at the beginning of his experiments. This may have been another manifestation of "laboratory diuresis" and salt loss. He found evidence for absorption of chloride, bromide, sodium, and, in one case, calcium ions. His experiments gave no absorption of iodide, nitrate, cyanide, potassium, and calcium ions (except for the case just mentioned). Sexton and Meyer (1955) have demonstrated the absorption of lithium as well as sodium

(Meyer, 1951) by the gills of goldfish, but no potassium, cesium, or rubidium ions were absorbed.

The graphic presentation of osmotic regulation in Fig. 2 is based on Baldwin's illustration (1948), and pictures the total process in fresh-water fish. The salt ions have been added to the original illustration to

TABLE IV
CHLORIDE ABSORPTION THRESHOLD FOR SOME SPECIES OF FISHES [a]

Species	Chloride concentration (m*M*)	Reference
Lampetra fluviatilis	0.00–0.05	Wikgren (1953)
Rutilus rutilus	0.00–0.05	Krogh (1937)
Carassius carassius	0.00–0.05	Wikgren (1953)
Carassius auratus	0.00–0.05	Meyer (1948)
Carassius auratus	0.10–0.20	Krogh (1937)
Perca fluviatilis	0.10–0.20	Wikgren (1953)
Gasterosteus aculeatus	0.20–0.40	Krogh (1937)
Platichthys flesus	0.20–0.40	Wikgren (1953)
Ameiurus sp.	0.40–0.80	Krogh (1937)
Acerina cernua	0.80–1.60	Krogh (1937)
Perca fluviatilis	>1.60	Krogh (1937)

[a] From Wikgren (1953).

TABLE V
RATE OF RELATIVE CHLORIDE ABSORPTION FOR SOME SPECIES OF FISHES [a]

Species	Weight g.	Rate of Cl Absorption (μM/100 g./hr.)	Reference
Lampetra fluviatilis	40–100	90	Wikgren (1953)
Salmo irideus	8	30	Krogh (1937)
Carassius auratus	48	27	Krogh (1937)
Carassius carassius	20–50	20	Wikgren (1953)
Rutilus rutilus	100	4	Krogh (1937)
Rutilus rutilus	49	16	Wikgren (1953)

[a] From Wikgren (1953).

indicate the ions which are known to be absorbed. It must be emphasized, however, that ion absorption may be specific; relatively few species have been examined.

Evidence for an interesting analogy between gill and kidney tubule action has recently been presented by Meyer (1952) and Sexton and Russell (1955) in Meyer's laboratory. Mercuric ion is known to produce a diuretic effect by upsetting normal sodium chloride and water reab-

sorption in the mammalian kidney. Meyer demonstrated that mercuric ion (10^{-5} *M*) completely inhibited the active uptake of sodium at the gills in goldfish, and increased sodium loss from 0.41 mg./100 g./hour to 4.02 mg./100 g./hour. A concentration of 10^{-6} *M* depressed sodium uptake 25% and increased sodium loss 43% over controls (normal uptake, 0.92 mg./100 g./hour; loss, 0.41 mg./100 g./hour.) It is known that the activity of succinic dehydrogenase in the kidney tubules of the dog and rat is depressed by the mercuric ion and consequently this enzyme may be involved in reabsorption of sodium chloride and water from kidney

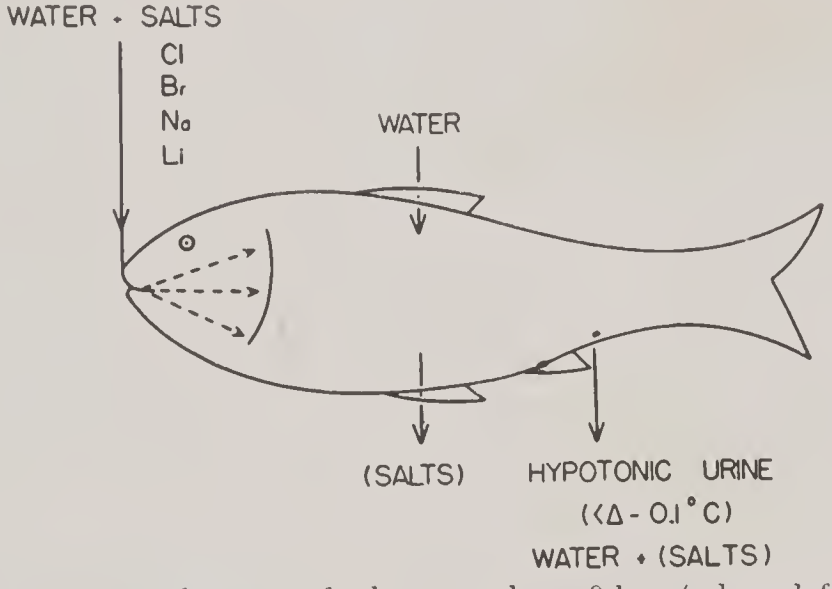

FIG. 2. Osmotic regulation in freshwater teleost fishes (adapted from Baldwin, 1948).

tubules (Handley and Lavik, 1950). Sexton and Russell (1955) determined the succinic dehydrogenase activity of goldfish gill filaments and found that 10^{-3} *M* mercuric chloride reduced oxygen uptake 59.5%. This work supports "the theory that succinic dehydrogenase is involved in active sodium transportation."

4. Transfer to Salt or Seawater Solutions

Many investigators (see Black, 1951) have tried to acclimate freshwater fish to more concentrated media by gradually increasing the salt content of the water. They have measured the changes in the concentration of the blood, urine, and/or tissues and have noted the viability of various species in salt water. Carp and goldfish (13, 14 of Table I) are particularly suited to such investigations for these species can live indefinitely in water with freezing point depressions down to —0.9° C. (about one-half sea water concentration).

Recent work by Takemura (1951) provides some interesting results

for changes in blood and subcutaneous tissue fluid of the loach (*Cobitis*), carp (*Cyprinus carpio*), and crucian carp (*Cyprinus cyprinus*) in sodium chloride solutions between 0.7% and 1.4%. Changes in blood and tissue fluid paralleled each other closely. An experiment on the carp (in a rather restricted position, and anesthetized with urethane) in 1.2% sodium chloride (Δ —1.3° C.) showed a gradual increase in tissue chloride from 205 mg.% to nearly 500 mg.% in 5 hours. When the solution bathing the gills was changed to tap water the tissue chloride fell to normal (205 mg.%) within an hour. Takemura suggests that control of permeability to salts may be effected by hormones and the autonomic nervous system.

In summary, it appears that the ability of stenohaline freshwater fishes to survive in salt solutions may depend on the histology of the gills, the extent of gill surface, rate of oxygen consumption, the tolerance of the tissues for salts, and control of permeability. The control of permeability may be the result of neurosecretory or hormonal reaction to the new environment, and it may also be the result of a direct effect on the surface cells. The latter condition can hardly be evaded. An ambient solution of balanced salts would presumably prove less harmful to the cells of the gills and oral membranes than a pure salt solution, since this has been repeatedly shown to be true for single cells. According to Heilbrunn (1952) calcium ion is of particular importance and is essential for the maintenance of normal cells.

Species differences in ability to tolerate salinity changes are too great to be covered by a single explanation, but the factors mentioned above must provide the limitations, to a greater or lesser extent, for stenohaline freshwater fishes.

B. Marine Fishes

1. Nitrogenous Excretion

The blood of marine fishes contains trimethylamine oxide which is excreted, in part, by the kidney. This substance is derived from the food of the fish and so is exogenous in origin and not a product of protein metabolism. Trimethylamine oxide, a weak base, may help to maintain the osmotic pressure of the blood, although Smith (1936) believes that its retention in teleosts is necessitated by the slow rate of urine formation. The urine of elasmobranchs contains only 10% of the concentration of trimethylamine oxide present in the blood (Hoppe-Seyler, 1930) which indicates conservation in this group (See also Section VI).

a. Cyclostomes. The kidneys of marine cyclostomes (hagfishes) are primitive segmented mesonephros (opisthonephros), each segment bearing a kidney tubule and glomerulus. The paired pronephroi with nephros-

TABLE VI

CONTENTS OF URINE OF NORMAL AND DIURETIC MARINE FISHES

Urine Constituents and Characteristics	Normal	Diuretic	Time after Capture	Reference
FREEZING POINT DEPRESSION				
Lophius piscatorius (aglomerular)	−0.68	−0.83		Grollman (1929)
Lophius piscatorius	−0.57	−0.76	After 53 hr.	Forster (1953)
Myoxocephalus octodecimspinosus (glomerular)	−0.62	−0.67	After 12 hr.	Forster (1953)
CHLORIDE (m*M*/l.)				
Opsanus tau (aglomerular)	86	165	In 2 days	Grafflin (1931)
Lophius piscatorius	56	188		Grollman (1929)
Lophius piscatorius	2	98		Pitts (1934)
Lophius piscatorius	12.3	242	After 53 days	Forster (1953)
Myoxocephalus octodecimspinosus	0	301	After 7 days	Grafflin (1931)
Myoxocephalus octodecimspinosus	0–29	169–285		Clarke (1934)
Myoxocephalus octodecimspinosus	0	147		Pitts (1934)
Myoxocephalus scorpius (partially glomerular)	0	124		Pitts (1934)
MAGNESIUM (m*M*/*l.*)				
Lophius piscatorius	38	108		Grollman (1929)
Lophius piscatorius	38	137		Pitts (1934)
Lophius piscatorius	103	243		Brull and Cuypers (1954a)
Myoxocephalus octodecimspinosus	66	161		Pitts (1934)
Myoxocephalus scorpius	74	72		Pitts (1934)
NITROGEN (mg.%)				
Total Nitrogen				
Myoxocephalus octodecimspinosus	107	11		Grafflin (1931)

Nonprotein nitrogen				
Lophius piscatorius	337	56		Grollman (1929)
Lophius piscatorius	210–255	140 (19–346)		Brull and Nizet (1953)
Creatine				
Lophius piscatorius	645	137		Pitts (1934)
Myoxocephalus octodecimspinosus	108	35		Pitts (1934)
Myoxocephalus scorpius	122	26		Pitts (1934)
URINE FLOW (ml./kg./day)				
Opsanus tau	3.6	11.2	After 2 days	Grafflin (1931)
Lophius piscatorius	29	75	After 53 hr.	Forster (1953)
Myoxocephalus octodecimspinosus	10, 4	71	After 6 days	Grafflin (1931)
Myoxocephalus octodecimspinosus	3.3	31–80		Clarke (1934)
Myoxocephalus octodecimspinosus	9.3	85.6		Forster (1953)
Myoxocephalus scorpius	2	40		Forster (1953)

tomes persist in the adult, but are probably not functional. As in marine teleosts (Section II, B,1,c) there is no distal convoluted tubule. The segmental glomeruli filter water, salts, and nitrogenous substances from the arterial blood as already described (see pp. 170, 171, 176). The capillary bed around the tubule is supplied with blood from the efferent glomerular artery; there is no renal portal vein such as occurs in elasmobranchs and teleosts.

Reports of investigations of excretion in cyclostomes seem to be rare and conflicting. Borei (1935) reported 350 mg.% urea in the blood of *Myxine;* Smith (1932) found 7–13 mg.%. The latter low range has been confirmed by Robertson (1954) who gives values of 4 and 2 m*M* urea in the blood of two specimens of *Myxine.* Possibly the nature of the host, whether elasmobranch or teleost, would influence the nitrogen content and excretion of hagfishes.

b. Elasmobranchs. The role of urea in all aspects of elasmobranch physiology has been reviewed by Smith (1936). Urine is formed as a glomerular filtrate and it has been assumed that most of the urea is reabsorbed in special tubule segments which lie at either end of the proximal convoluted tubule. Kempton (1943), however, was able to identify typical vertebrate segments in the tubules of the spiny dogfish, *Squalus acanthias.* Apparently the cytology of the kidney tubules of elasmobranchs requires further investigation.

The permeability of the gills to urea is much reduced, compared with that of teleosts, so that most of the urea is retained permitting an osmotic concentration that is slightly greater than that of sea water. Smith (1953) states that the gills of elasmobranchs and the renal tubules of mammals are the only cell structures known to be virtually impermeable to urea. In addition to urea, trimethylamine oxide assists in maintaining the high osmotic pressure of the blood, and is responsible for about one-quarter of the osmotic pressure produced by organic substances (Smith, 1936).

The blood of marine elasmobranchs contains 2–2.5% urea; in other vertebrates blood urea amounts to only 0.01–0.03% (Smith, 1936). Kempton (1948) has found that the urea blood level of the dogfish, *Mustelus canis,* is highly variable (745–2100 mg.%). He reported (1953) that 70–99.5% of the urea filtered through the glomeruli may be reabsorbed by the kidney tubules; thiourea, however, is reabsorbed to only a slight extent (Clarke and Smith, 1932). The site of urea reabsorption in relation to the site of water reabsorption could not be determined by Kempton's experiments, but the data showed that reabsorption of urea is not an isosmotic process. The amount of unabsorbed urea is correlated inversely with the reabsorption of water, and directly with the rate of urine flow and concentration of urea in the urine.

Some analyses of non-protein nitrogen in the urine of elasmobranchs are given in Table II. The average urea content of the blood is 1000–2000 mg.%; of the urine, about 230 mg.%. Kempton (1953) reported an average plasma urea nitrogen of 898 mg.% for 10 specimens of *Mustelus canis;* the average urea nitrogen in the urine was 396 mg.%. Although data for uric acid, amino aeids, creatine, and creatinine are not given for urine, these substances have been found in the blood of marine elasmobranchs (Denis, 1922), and are presumably excreted in the urine. The amino acid and creatine content of the blood were significant and higher than in teleosts.

Elasmobranchs now living in fresh water are thought to have reinvaded this medium from the sea, because they retain a high blood urea content. The excretion of nitrogenous wastes in freshwater elasmobranch fishes has been studied by Smith and Smith (1931) and their work has been reviewed by Krogh (1939). They showed that in *Pristis* 89% of the total ammonia exereted was given off extrarenally. There appeared to be some physiological regulation of the ammonia given off (presumably by the gills), but the urea (67% of which was excreted extrarenally) left the fish by diffusion. Average data for urine flow and nitrogen content in *Pristis* are given in Tables II and III.

c. Teleosts. Edwards (1935) reported that his eytological examination of kidney tubules in marine teleosts showed that the distal eonvoluted tubule is normally absent, and the neek segment eonnecting tubule with glomerulus is eonstricted in some marine teleosts, although open in freshwater fishes. The proximal eonvoluted tubule in all glomerular teleosts is divided into two parts, the second part being homologous with the tubule in aglomerular fishes (Seetion II, B, 2, d).

Most of the nitrogen in marine teleosts is excreted extrarenally, as in freshwater fishes. Small amounts of ammonia and urea, however, appear in the urine with the trimethylamine oxide mentioned above. The nitrogen excretion of the aglomerular fish, *Lophius piscatorius,* has been investigated thoroughly. Since the nephron of this species has no functional glomerulus, nitrogenous substances reach the lumen of the tubule by secretion of the tubule cells. Brull, Nizet, and Verney (1953) found that nonprotein nitrogen was actively coneentrated in the urine secreted by perfused kidneys. Aecording to Grollman (1929) ammonia composes less than 1% of the total urinary nitrogen; the urea, uric acid, and creatinine are also low (Table II). Amino acids appeared in about the same concentration as in the blood, and ereatine was present in large amounts. These substances comprised about 50% of the nitrogen in the urine. The first identification of trimethylamine oxide in fish urine was made by Grollman (1929) in *Lophius.* Sinee then Baldwin (1948) and Brull and Nizet (1953) have reported, respeetively, that 28% and

20–50% of the urinary nitrogen is in the form of trimethylamine oxide. Brull and Nizet (1953) found that they still could not account for 30% of the nonprotein nitrogen in the urine in early experiments, but analysis of pooled *preserved* urine showed that 90% of the nonprotein nitrogen was made up of creatine, ammonia, and trimethylamine oxide (Brull and Cuypers, 1954a). Grollman's determinations of nonprotein nitrogen at the time of capture were as much as six times the amount present in fishes kept in aquaria. Inorganic constituents, on the other hand, increased after capture (Table VI).

2. Water Balance

a. Cyclostomes. Since the concentration of the blood of the hagfishes is nearly isotonic with the external environment (Section II, B, 3, a), water movement due to differences in osmotic concentration is low. Most of the water required for urine formation is obtained from the tissues and blood of the fishes to which these animals attach themselves.

For discussion of *Petromyzon* see Section III.

b. Elasmobranchs. The blood of elasmobranchs is maintained at a concentration which slightly exceeds that of the surrounding sea water (see Table I, and Fig. 4). By this means an osmotic intake of water across the gills and oral surfaces is assured and elasmobranchs do not have to contend with dehydration as do the marine teleosts. They rarely drink sea water, and obtain free water in their food.

All elasmobranchs have glomerular kidneys and, as a rule, excrete urine that is slightly hypotonic to the blood. The extent of hypotonicity appears to be related to the concentration of the sea water, i.e. in fish in dilute sea water the difference in concentration between blood and urine is greater (7 of Table I). The urea cycle in elasmobranchs is responsible for maintaining the water balance as described by Smith (1953); "as the water absorbed through the gills dilutes the blood, the urine flow increases, increasing the excretion of urea and thereby reducing the blood urea concentration, which in turn reduces the urine flow, and starts a new cycle of urea retention, so that the animal is always supplied with enough free water to meet its urinary requirements."

Urine flow in marine elasmobranchs is low (Table III) varying between 2 and 24 ml./kilogram/day. The average daily glomerular filtrate is 80 ml./kilogram (Smith, 1936), the difference between filtrate and flow is the amount reabsorbed in the kidney tubule.

c. Glomerular teleosts. Marine teleosts tend to lose water continually across the gills and other body surfaces, because the concentration of

the blood (approximately Δ —0.78° C.) is lower than that of the sea water (Δ —1.8–2.2° C.). In order to replace the water lost osmotically, the fishes swallow sea water, and also gain water in their food. Smith (1930a) found that about 78% of the sea water swallowed (50–200 ml./kilogram day) by the eel (*Anguilla rostrata*) was absorbed; the sculpin (*Myoxocephalus*) absorbed about 63%. His experiments also indicated that about 59% of the water is lost extrarenally in the eel, 31% in the sculpin.

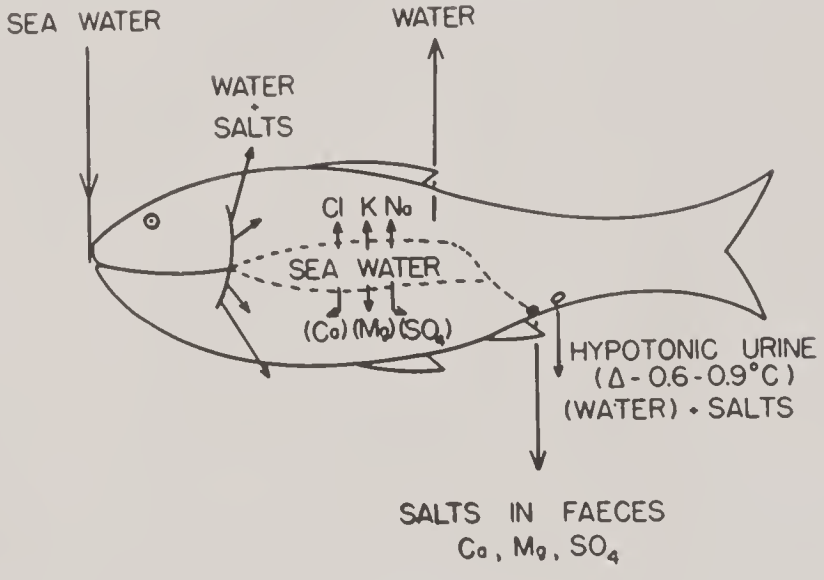

FIG. 3. Osmotic regulation in marine teleost fishes (adapted from Baldwin, 1948).

Water reabsorption takes place in the kidney tubule (Marshall and Grafflin, 1932). In *Myoxocephalus octodecimspinosus,* 14 ml. fluid are filtered through the glomeruli, 3 ml. of which are excreted (per day) as urine (Clarke, 1934). The relation of glomerular filtrate and urine flow in glomerular *M. octodecimspinosus* and partially glomerular *M. scorpius* is well described by Forster (1953) for normal and diuretic fishes. The small amount of urine excreted by marine teleosts, though hypotonic, is very near the concentration of the blood because most of the water has been reabsorbed (see Table I and Fig. 3).

d. Aglomerular teleosts. The presence of aglomerular and partially glomerular kidneys in marine teleosts is of particular interest as evidence for the theory that structures no longer needed tend to disappear during the course of evolution. Because of the osmotic water loss by marine teleosts there are not large quantities of water passing through the glomeruli to form copious urine as in freshwater fishes; hence glomeruli

in marine species appear to be less numerous (*Myoxocephalus scorpius*), present in small numbers but nonfunctional (*Lophius piscatorius*), or completely absent (*Opsanus tau*). The extent of distribution of glomerular and aglomerular kidneys among marine teleosts could be further investigated, especially in view of the fact that the glomerular status appears to be a species characteristic (*M. octodecimspinosus* fully glomerular, *M. scorpius* partially glomerular).

Studies of the structure (Edwards, 1928, 1929; Grafflin, 1937a, b; Nizet and Wilsens, 1955) and function (Edwards and Condorelli, 1928; Marshall, 1930) of the aglomerular kidney of certain marine fishes have proved especially useful in determining the functions of the glomerular and tubular parts of the kidney, because the aglomerular tubule corresponds to the second segment of the proximal convoluted tubule. Hence the functions performed by the aglomerular kidney may be assigned to the proximal convoluted tubules in glomerular species. Fish without functional glomeruli seem to be able to carry out all the necessary processes for excretion. This fact, and other investigations, have led to the conclusion that the glomerulus serves only as a filter for water, salts, nitrogenous substances, and sugar which enter the kidney tubule from the capillaries of the glomerulus, and which may be reabsorbed or excreted later. In aglomerular fishes the tubule is supplied with venous blood from the renal portal vein. The blood is collected again from the capillary circulation and enters the post-cardinal vein. There is no appreciable supply of arterial blood such as is received by the glomerulus in glomerular fishes. The substances to be excreted from the blood, including small amounts of water, are secreted into the kidney tubule (Brull and Cuypers, 1954a; Marshall and Grafflin, 1932). Brull and Cuypers (1954b) and Brull (1955) have shown by perfusion experiments that the secretion of urine in *Lophius* is dependent on the blood pressure when the latter is below 100–225 mm. of water. Pressures above 225 mm. do not affect the urine flow, i.e. the tubular maximum has been reached. They concluded from their experiments that water secretion by the aglomerular kidney is an active process, since urine is secreted at 30–40 mm. of water when the colloid osmotic pressure of the blood is 85 mm.

The two marked physiological differences between the glomerular and aglomerular kidneys are: (1) inability of the aglomerular kidney tubule to excrete sugars, (2) ability of the aglomerular kidney to secrete against a higher pressure than the blood pressure. Many studies have been published on tubular excretion of various substances and dyes. Shannon (1938) showed that phenol red, which is excreted by the aglomerular kidney, cannot diffuse across the tubule, but must be carried by active transport.

Water and salt balance in teleost marine fishes is pictured in Fig. 3. The substances in brackets are secondary in the process of osmotic regulation. Salt ions actively excreted by the gills are univalent (Na, Cl), those actively excreted by the kidneys are divalent (Ca, Mg, SO_4).

3. Salt Balance

a. Cyclostomes. The marine genera, *Myxine* and *Polistotrema* (hagfishes), have a body fluid concentration closely comparable to that of the environment. In *Myxine* (3 of Table I) the values reported by

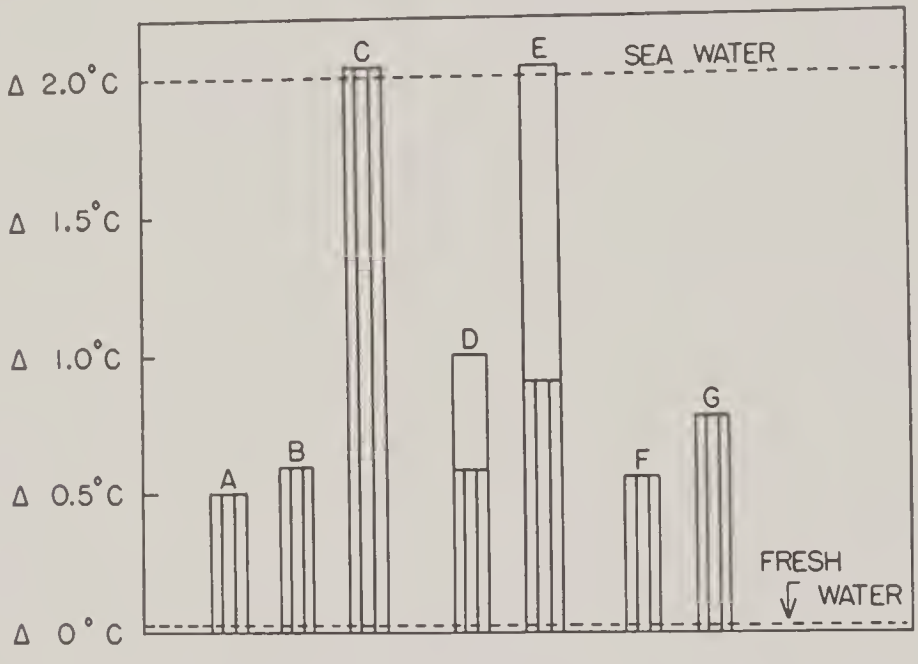

Fig. 4. Osmotic pressures of bloods of fishes compared with those of fresh and sea waters. A: lampreys in fresh water; B: lampreys in sea water; C: hagfishes; D: freshwater elasmobranchs; E: marine elasmobranchs; F: freshwater teleosts; G: marine telesosts. Base-line sections, salts; top sections, urea (adapted from Baldwin, 1948).

Smith (1932) are slightly hypertonic to the sea water. The Schmidt-Nielsens (1923) found that within a few hours the blood of *Myxine* became isotonic to sea water between Δ −2.32 and Δ −1.85. In contrast to the clasmobranchs (Section II, B, 3, b), however, the high concentration is due largely to salts in the blood, and so these fish (hagfishes) are considered poikilosmotic. With body fluids practically isotonic with the surrounding water, osmotic regulation presents no problem. Recent experiments by Robertson (1954) on *Myxine* serum have shown that inorganic ions account for 99.7% of the osmotic concentration, and that the concentration of each ion is specifically regulated. Bond, Cary, and Hutchinson (1932) found that there was always slightly less chloride in the blood of *Polistotrema* than in the sea water (ratio 0.89).

For discussion of *Petromyzon* see Section III.

b. Elasmobranchs. Osmotic regulation in marine elasmobranchs requires active ion transport to maintain the proper salt balance. Chloride, magnesium, sulfate, and phosphate may appear in the urine, but much of the salt content in the glomerular filtrate is reabsorbed by the kidney tubules. The actual salt content of the blood of marine elasmobranchs is only slightly higher than that of marine teleosts (Fig. 4), but the increase in blood osmotic pressure (due to the presence of large amounts of urea) to a concentration greater than that of the surrounding sea water, eliminates the regulatory problems faced by the marine teleost.

Clarke (1936) could not induce "laboratory diuresis" in the dogfish (*Squalus acanthias*) "under ordinary experimental conditions." However, there are data (Table III; Smith, 1931; Clarke and Smith, 1932) which show urine flows up to 56 ml./kilogram/day in dogfish and rays, and also the high urine chloride and magnesium characteristic of "laboratory diuresis." In some cases the chloride in the urine exceeded that in the plasma and certainly indicated an upset in salt balance.

The function of carbonic anhydrase in the kidney and gills of the marine dogfish (*Squalus acanthias*) has been investigated recently by Hodler *et al.* (1955). The manuscript reporting this work was made available to the author through the kindness of Dr. Smith. The carbonic anhydrase inhibitor, Diamox, was injected intravenously and caused no change in the pH of the urine, so that the kidney in dogfish does not appear to be dependent on carbonic anhydrase for rapid and continuous supply of hydrogen ions. In the gills, however, carbonic anhydrase appears to function in the excretion of bicarbonate ions and CO_2 following the injection of $NaHCO_3$. When the inhibitor, Diamox, is also injected there is no further increase of HCO_3 excretion. The authors point out that "these experiments on acid–base balance throw no light on the mechanism by which sodium chloride is excreted by" the gills of "marine fish." However, knowledge of the facility of excretion of bicarbonate ion by the gills of these fish is an important contribution to the physiology of fishes.

The salt concentration of the blood of freshwater elasmobranchs is close to that of freshwater teleosts (Fig. 4), the high total osmotic pressure being caused by the uremia characteristic of elasmobranchs.

c. Glomerular teleosts. Marine teleosts with a total blood concentration near Δ —0.78° C. have to swallow sea water to prevent dehydration caused by the osmotic outflow of body fluid water toward the more concentrated sea water. In order to maintain the low body fluid concentration, however, the sea water salts must be excreted. Smith (1930a) first suggested that the gills were responsible for the excretion of salts,

and Keys (1931a) found evidence of the active secretion of salts into the water by special cells located in the gills of the eel, *Anguilla anguilla*. It is this mechanism of salt excretion at the gills which makes marine life possible for fish maintaining a low osmotic pressure in the blood. Keys and Willmer (1932) were unable to find these special cells in the gills of the elasmobranch *Scyliorhinus*. This is reasonable since elasmobranchs do not need to secrete salts (Section II, B, 3, b).

Important work on the histology of salt-secreting cells in the gills of the euryhaline fish, *Fundulus heteroclitus*, has been published by Copeland and his associates (1948, 1950; Pettengill and Copeland, 1948; Getman, 1950) (see Section V). Of particular interest here is the discovery of these cells in the oral membranes as well as in the gills, a large number being located on the inner surface of the operculum (Burns and Copeland, 1950).

The nature of these cells and their true function has not been unconditionally accepted. Bevelander (1935, 1936) examined 36 species of fish including freshwater and marine cyclostomes, elasmobranchs and teleosts. He concluded that the "secretory" type cell was a mucous cell and that the exchange of ions at the gills took place across the respiratory epithelium. His interpretation is supported by the fact that he found no correlation between the nature of the environment and the number and size of the "mucous" cells. On the other hand, the majority of species examined had respiratory epithelium composed of squamous cells, and it is difficult to see how these could perform the osmotic work required to secrete against a gradient.

Of the salts swallowed in the sea water, sodium, potassium, and chloride ions are absorbed from the gut. These ions do not normally appear in quantity in the urine but are excreted by the gills. Most of the calcium, magnesium, and sulfate ions become concentrated in the residual alkaline intestinal fluid, and the cations are eliminated as insoluble oxides or hydroxides. Small amounts of these ions, however, are also absorbed and excreted in the urine. The importance of the acidity of the urine is obvious here, since in neutral and alkaline solutions insoluble salts would be formed as in the intestine (Smith, 1953).

In vitro experiments on transport of substances in isolated kidney tubules of the flounder (*Pseudopleuronectes americanus*) were successfully carried out by Forster (1948) and this technique has been used to study the transport of dyes (Forster, Sperber, and Taggart, 1954), and to determine the forces responsible for active transport of substances by the kidney. The latter is of particular interest and Taggart and Forster (1950; Forster and Taggart, 1950) were able to show that phosphate bond energy, oxygen, cytochrome oxidase, and dehydrogenases are

essential for phenol red transport in the flounder (*P. americanus*). Browne, Pitts, and Pitts, also in 1950, demonstrated the presence of alkaline phosphatase in 10 species of marine teleosts, 3 of which were aglomerular, but not in the eel. This enzyme had been thought to be characteristic of glomerular teleosts where it functioned in the reabsorption of glucose from the glomerular filtrate (Wilmer, 1944). Its presence in aglomerular fishes where there is no excretion of sugar, indicates that the enzyme has other functions as well. If the isolated tubule technique can be applied to the eel it would be interesting to discover whether or not 2,4-dinitrophenol, which inhibits utilization of phosphate bond energy (Taggart and Forster, 1950), has an effect on its tubular transport.

The effect of salt ions on tubular transport of phenol red in the flounder (*P. americanus*) kidney has been studied by Puck, Wasserman, and Fishman (1952) and Wasserman, Becker, and Fishman (1953), using an adaptation of Forster's technique. They found that the concentrating power of isolated tubules decreased as the external concentration was increased. In tubules bathed in solutions low in potassium and high in calcium, phenol red accumulated in the lumen; none was seen in the cells. In high potassium with no calcium the reverse was true. Without potassium there was no uptake of the dye in cells or lumen. It was suggested, therefore, that the absorption into the cell requires potassium and secretion from cell to lumen requires calcium. The inhibition of carbonic anhydrase by Diamox was found to facilitate phenol red accumulation in tubule cells and prevent acidification of tubular urine.

In vivo, Heinemann and Hodler (1953) found that Diamox produced no significant changes in the normal urine excretion of *Squalus, Myoxocephalus, Lophius,* or *Micropterus,* indicating that carbonic anhydrase is not involved in acidification of urine in these fishes. In the catfish (*Ameiurus nebulosus*), however, with Diamox the urine pH increased from 6.43 to 7.34 and large increases also occurred in sodium and potassium excretion and in urine flow. Similar but smaller changes were observed in the flounder (*P. americanus*). In these two species carbonic anhydrase must be involved in urine acidification.

d. Aglomerular teleosts. The salt contents of the body fluids of *Lophius,* the goosefish or angler, have been thoroughly studied, especially in connection with "laboratory diuresis." Grollman (1929), Brull and Cuypers (1954a), and Forster (1953) found low urine chloride in goosefish taken immediately from the sea. After being transferred to aquaria, however, the chloride in the urine increased and the osmotic pressure of the urine began to approach that of the plasma, which was also rising. The loss of magnesium in aquarium fish is also very high. Grollman (1929) and Brull and Cuypers (1954a, 1955) report a threefold

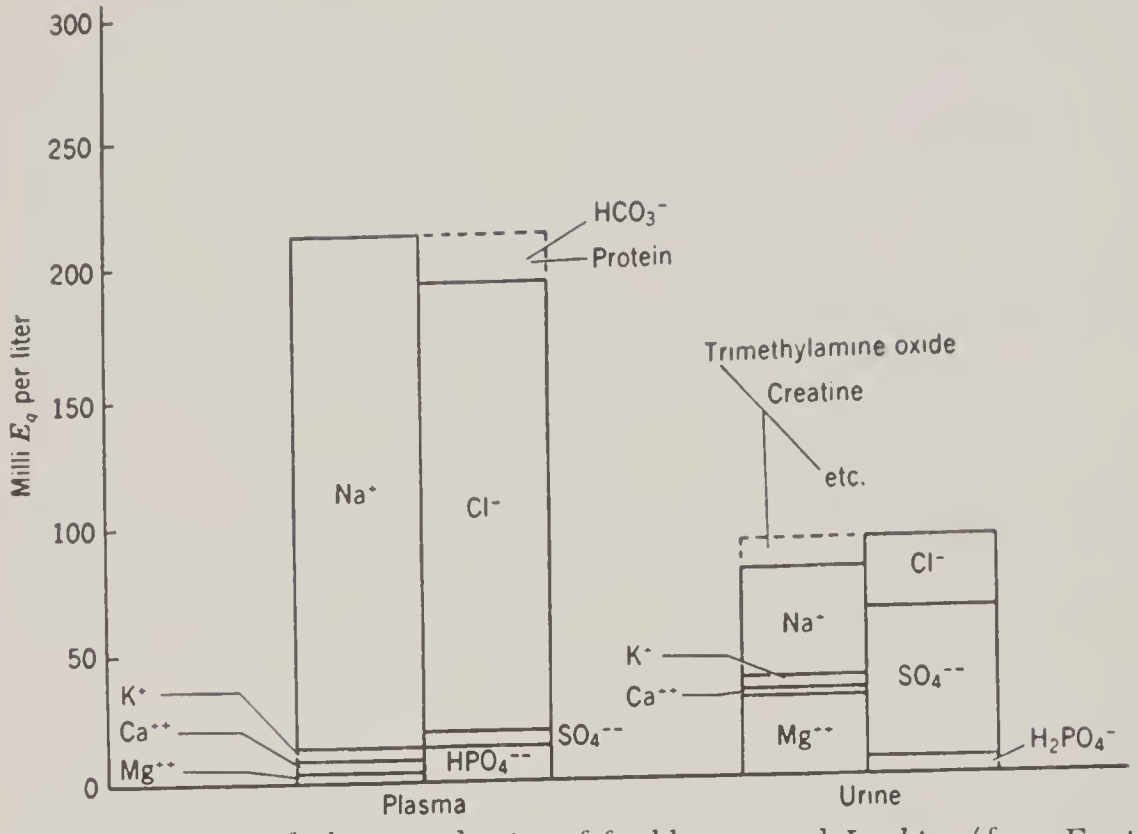

FIG. 5. Contents of plasma and urine of freshly captured *Lophius* (from Forster and Berglund, 1953).

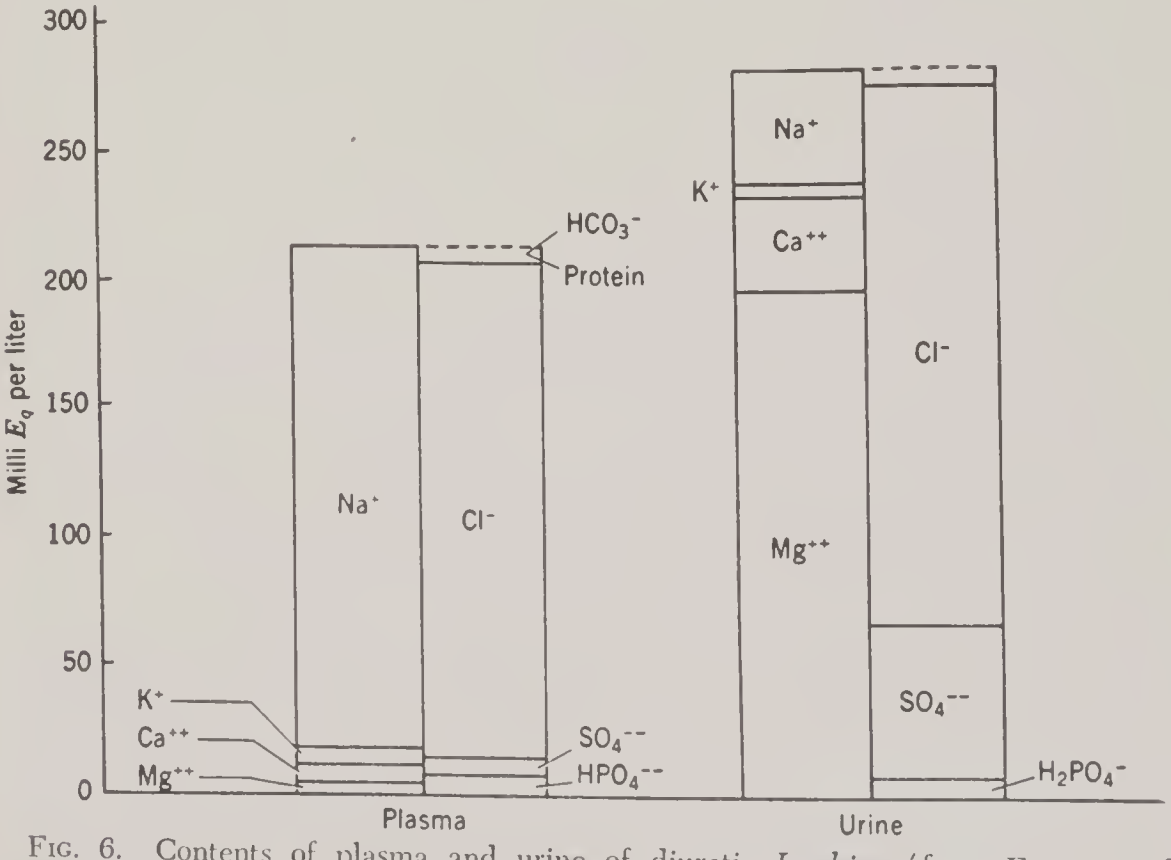

FIG. 6. Contents of plasma and urine of diuretic *Lophius* (from Forster and Berglund, 1953).

increase in the magnesium in the urine and plasma after capture. The increase in salt content and urine flow in fish with "laboratory diuresis" indicates abnormally high ingestion of sea water. Clarke (1934) showed that diuresis does not occur when the entrance to the gut is blocked, so that sea water cannot be swallowed. Forster (1953) found that the filtrate in a glomerular fish (*Myoxocephalus octodecimspinosus*) increased from 14 to over 100 ml./kilogram/day. A previously unpublished analysis of the constituents of plasma and urine in normal and diuretic *Lophius* (Figs. 5 and 6) has been made available by the kindness of Dr. Forster (Forster and Berglund, 1953). Figures 5 and 6 show that urinary constituents change markedly and selectively during diuresis. A comparison of the urine constituents of normal and diuretic marine teleosts is given in Table VI. The decrease in urine nitrogen during diuresis may indicate that in the presence of high salt content this substance could not be properly metabolized, or it may just reflect the fact that feeding and digestion cease during this physiological crisis.

4. Transfer to Dilute Sea Water or Fresh Water

According to Smith (1953) experimental transference of marine elasmobranchs to fresh water is rarely successful, although they are sometimes found in nature in fresh river water. Several species (Table I) survive indefinitely in dilute sea water, and show a marked increase in urine flow. According to Dakin (1935) Duval found that in dogfish transferred from Δ —2.08 to Δ —1.07° C., the blood concentration decreased from Δ —2.17 to Δ —1.76° C.; 70% of this decrease was due to fall in urea content, 25% due to fall in chloride content.

Henschel (1936) showed that of two species of flounder (teleosts) *Pleuronectes* (= *Platichthys*) *flesus* can adapt to dilute sea water better than *Pleuronectes platessa* (27, 28 of Table I); the former stops drinking water, increases urine excretion, and retains body salts. Wikgren (1953) presents evidence that *P. flesus* can absorb ions from fresh water, and loses only small amounts of salt through the skin (Tables III, IV).

Recently Forster (1953) found that when *M. octodecimspinosus* is maintained in dilute sea water the urine flow eventually exceeds the glomerular filtration rate as measured by inulin clearance.

Two species of aglomerular fish are known to frequent tidal or fresh waters. The toadfish (*Opsanus tau*) penetrates the creeks of Chesapeake Bay and the freshwater pipefish (*Microphis boaja*) breeds in tropical rivers (Smith, 1953). They apparently can make adequate osmotic adjustments without the help of glomeruli (Grafflin, 1937c). A study of the physiology of excretion and osmoregulation in these fishes in fresh water should prove most rewarding.

Many investigators (see Black, 1951) have noticed that the presence of calcium salts in fresh water greatly increases the viability of marine and euryhaline animals in that medium. One reason for this effect is the well-known fact that calcium decreases cell permeability, both to salts and water.

Salt concentrations greater than sea water (Δ —2.85 to —3.0° C.) are eventually lethal to marine teleosts when the capacity of the gills to secrete salts is exceeded and salts accumulate in the tissues (Leövey, 1938) (21 of Table I).

III. ANADROMOUS FISHES (SALMON AND LAMPREY)

The following section concerns fishes, like the salmon and lamprey, that migrate from the sea to spawn in fresh water. The salmon spends its adult life in the sea, and migrates up the rivers to spawn. The eggs develop into fry or large fish, depending on the species, before the return to the sea is undertaken. The literature concerned with the osmoregulatory aspects of salmon migration has been recently reviewed by Fontaine and Koch (1950) and Black (1951).

It is significant to realize that diadromous (anadromous and catadromous) fishes are not necessarily euryhaline, but are able to migrate because of hormonal activity (see Chap. VI) that results in the physiological changes required for survival in the new environment. Salmon eggs, for instance, cannot develop normally in saline solutions. Young salmon (*Oncorhynchus kisutch*) cannot enter the sea until salt-secreting cells have developed in the gills (Hoar, 1949), and other physiological changes have taken place. Nishida (1953) has also found cytohistological differences in the secretory cells of the gills of parr and smolt of *Oncorhynchus masou*. When returning to fresh water, adult salmon exhibit a slight decrease in the osmotic concentration of the blood (10, 12 of Table I).

Some lampreys (cyclostomes) are also anadromous. *Petromyzon marinus* (2 of Table I) spends some time in sea water but returns to the rivers, and dies after breeding. Some species are found only in fresh water and migrate from lakes to streams (potamodromous).

Data presented in Table I show that osmotic concentration of the body fluids is comparable to that of teleosts, and consequently active osmotic regulation must take place. Wikgren (1953) made a careful study of osmotic regulation in *Lampetra fluviatilis* in fresh water. He found that at 18° C. the daily urine output is large (about 45% of the body weight), but the urine chloride content is low (0.5–1.5 mM) (Table III). The body surface is moderately permeable to water and considerable quantities of water are absorbed osmotically, because of the large area of

the branchial surface. The permeability to chloride is about 0.1 μM Cl/sq. cm./hour and the mechanism for chloride absorption from fresh water is well developed (Tables IV, V). *L. fluviatilis* survives well when transferred, by stages, to 16–20 ‰ sea water, or when directly transferred to 6 ‰ sodium chloride solution.

At temperatures near freezing (0–3° C.) permeability to water and probably to salts is lowered. Since lampreys do not feed at these temperatures, the lowered permeability and continued, though decreased, absorption of chloride by the gills help to maintain the salt balance. Wikgren's work shows that lampreys (*L. fluviatilis*) are well equipped to carry out the function of osmotic regulation.

The marine lamprey can regulate body fluids until the time of anadromous migration. Fontaine (1930) experimented with *P. marinus* after migration to fresh water had begun (April). He found that the fish could not tolerate even half sea water salinities. The serum freezing point depression was —0.54° C. and the chloride content was 6.86 g./l. NaCl in fresh water; these rose to Δ —0.77° C., chloride 8.50 g./l. in water of Δ —1.0° C., after a 3 day gradual increase in salinity. These results indicate again the stenohaline nature of anadromous fishes at certain times in the life cycle.

Recent work by Sawyer (1955) on *P. marinus* shows that fish migrating from the sea to fresh water assume very quickly the functions of typical freshwater fishes (2 of Table I; and Table III).

IV. CATADROMOUS FISHES (EEL)

Eels have been studied a great deal in connection with osmotic regulation in fresh and sea water. The following account is condensed from Krogh's book (1939) and reviews by Fontaine and Koch (1950) and Black (1951) and is limited to eels breeding in the Atlantic Ocean.

Eels (*Anguilla* spp.) migrate as adults from continental fresh waters to spawn in the Sargasso Sea in the Atlantic Ocean. The larvae (leptocephali) produced here drift about for 1 to 3 years as stenohaline marine animals. The larvae then metamorphose into young eels which become euryhaline and eventually enter rivers and fresh waters. These young fish (elvers) display good osmotic regulation when transferred, in stages, from fresh water to sea water (Δ —2.0° C.), since the concentration of the tissues remains below Δ —1.0° C. At concentrations higher than Δ —2.0° C., however, control of the internal concentration is lost. Adult eels can maintain an internal concentration less than Δ —0.8° C. when in sea water Δ —2.0° C.

The renal unit of the kidney of the eel contains glomerulus, neck segment, proximal and distal convoluted tubules, and initial collecting

tubule (Grafflin, 1937c). When transferred from fresh to salt water and the reverse, eels show significant changes in weight for several hours, presumably because of the osmotic flow of water into (in fresh water) or out of (in salt water) the fish. The quantity of water passing through the surface of the eel is remarkably small under these conditions; most of the exchange is by mouth or by urine excretion. Osmotic regulation then restores the normal water and salt balance (see Section V). In his work on absorption of chloride from the water by fish in fresh water, Krogh was unable to measure any absorption by adult eels (see Section V). However, he found that eels are able to retain chloride to a remarkable degree and concluded that, in fresh water, eels maintain their salt supply by absorbing salts from food and reabsorbing practically all chloride contained in the glomerular filtrate in the kidneys.

Osmotic regulation just before and during the migration of the fasting eel to the ocean breeding grounds has been studied in Fontaine's laboratory (Paris) especially by Callamand (1943). They suggest that loss of body salts is a cue for beginning to migrate to the sea where salts could be regained. However, the evidence correlating "demineralization" with migration is controversial. Hormonal activity at the time of migration is probably responsible for the adjustment in osmotic regulation. The migration of young eels (elvers) to fresh water seems to be facilitated by the activity of the pituitary gland (Vilter, 1946) since hypophysectomized elvers do not avoid sea water, whereas normal elvers do. Euryhaline adult eels, however, can adjust to salinity changes when they are hypophysectomized just as easily as can normal eels (Fontaine, Callamand, and Olivereau, 1949).

In comparative investigations the eel appears to have exceptional features. Krogh demonstrated chloride absorption by gills of all freshwater fish he examined, except the eel. Browne, Pitts, and Pitts (1950) demonstrated alkaline phosphatase activity in kidneys of several species of marine fish, but not of eels. Kobayashi and Yuki (1954) found that the eel kidney showed the lowest catalase activity of 35 species of marine, freshwater, and diadromous fishes examined. The salmonoids showed very high activity, freshwater fish low activity, and marine fish varied widely in kidney catalase activity.

V. EURYHALINE TELEOST FISHES

Euryhaline fishes are able to tolerate a range of salinity in the water. Although some such species do not appear to have any preference or motives in their movements, other species undertake short spawning migrations, usually to fresh or brackish water. Gunter (1942) found that for every freshwater fish that has been taken in sea water in North

America, nine species of marine fish have been taken in fresh water. It seems to be easier for fishes to adapt themselves to excess water than to excess salt.

The two genera that have been most frequently investigated in an effort to determine the anatomy and physiology of euryhalinity are *Fundulus* (Sumner, 1905; Scott, 1908; Grafflin, 1938; Black, 1948) and *Gasterosteus* (Gueylard, 1924; papers by Koch and Heuts, University of Louvain, 1942, 1943, and later papers). It is of interest that in both these genera (and in "*Pleuronectes*," see Section II, B, 4) species differ in their ability to adapt. *F. diaphanus* and *G.* (i.e. *Pygosteus*) *pungitius* are less able to tolerate sea water than *F. heteroclitus* and *G. aculeatus*.

In all cases where measurements have been taken of fish successfully transferred to a more concentrated environment there has been a temporary loss of weight (water), and increase in salt concentration. But the necessary physiological adjustment is made within 48 hours and weight and concentration return to normal.

When, on the other hand, fishes go from the sea to fresh water they gain water and lose salts and must accommodate by increased urine flow and salt retention.

The structures which permit adjustments to salinity changes may be listed as follows:

(1) Body surface permeability. In a successful migration either to or from a concentrated environment a low permeability of the body surface to water (and salts) is a great asset. On the other hand, permeable surfaces such as the gills may be an asset rather than a liability if they help make the adjustment to the new medium. For instance, Keys (1931b) and Gunter (1942) noted that small members of a species (with longer head length in relation to total length) survived dilution better than the large fishes, because the greater gill surface helped during temporary respiratory stress resulting from dilution.

(2) Kidney activity. A fish entering fresh water will need an increased glomerular filtrate and urine flow in order to dispose of the water entering across the body surfaces. Reabsorption of salts by the kidney tubule is important in fresh water. When invading the sea, urine excretion is automatically reduced because the body fluids become more concentrated due to osmotic water loss. The presence or absence of the distal convoluted tubule in euryhaline fishes is considered an indication of the medium of origin of the species, but does not seem to be a limiting factor in the adjustment. Even glomeruli do not appear to be essential for fishes in fresh water, for the aglomerular toadfish and pipefish are found in estuaries and rivers (Section II, B, 4).

(3) Gills. Interesting and valuable work has been done on the cytology of the gills in Copeland's laboratory. In *Fundulus* the same

type of cell may be responsible for both excretion and absorption of chloride in salt and fresh water (Copeland, 1948, 1950). Copeland (1950) found that the chloride cells of *F. heteroclitus* adapt more rapidly to fresh water (4 hours) than to sea water (7–9 hours). He has described the chloride cell as "a columnar, acidophilie cell found in the gill filament and localized on the side of the filament supplied with afferent blood" (1948). When excreting chloride an "excretory vesicle" is present at the free end of the eell. Copeland showed that the activity of the cell was determined by the concentration of the *internal* environment. Evidence for cell function in fresh water is based on the inereased phosphatase activity of the cell (Pettengill and Copeland, 1948). Getman (1950) obtained similar results when he examined the cells of the gills of the eel (*Anguilla rostrata*). However, all of the chloride excreting cellular pits of the cells of eel gills did not disappear when the eel was transferred to fresh water. Getman suggested that the reason Krogh could not demonstrate chloride absorption by the eel in fresh water might have been because a sufficient number of eells were still actively excreting chloridc to nullify the effect of those cells absorbing salts. The cytological change from sea to fresh water took place within 15 hours in the eel.

The center of control for the change in activity of these struetures and for other behavior, such as drinking sea water, but not fresh water, is still undetermined. Neural control has been suggested, and hormonal activity has been investigated to some extent. An active thyroid (as during migration and spawning) is detrimental to osmotie regulation in sticklebacks (Koch and Heuts, 1942) and eels (Fontaine and Callamand, 1943). The hypophysectomized adult eels tolerate sea and fresh water equally well (Section IV). Pickford (1953) found that *F. heteroclitus,* on the other hand, could not survive in fresh water or dilute sea water after hypophysectomy. An examination of the fishes showed the formation of kidney stones.*

In nature, salinity changes are usually gradual and the fish has some choice in the speed of transfer. In most experimental procedures, however, fishes are submitted to stress because of fairly sharp ehanges in salinity, handling, etc., and those that survive our experiments are indeed hardy breeds.

VI. CONCLUDING REMARKS

In reviewing nitrogen excretion in fishes, three substances have been found to hold special interest. They are urea, ammonia, and trimethylamine oxide. Urea is undoubtedly an endogenous product of protein

* Experiments by Burden (1956) indicate that hypophysectomy in *F. heteroclitus* results in atrophy of the gill mucous cells but does not affect the chloride cells.

metabolism in all fishes, and plays an active part in many phases of elasmobranch physiology. The large amounts of ammonia excreted by the gills of fish are thought by Smith (1953) to be formed at the site of excretion. Comparatively small amounts of this toxic substance are found in the blood and urine.

In marine fishes some nitrogen appears in the form of nontoxic trimethylamine oxide, a substance of exogenous origin which is more plentiful in the diet of fish living in the sea. An interesting study of the distribution of trimethylamine oxide in muscles of fish has been made by several investigators, and tables compiled by Shewan (1951) show that young king salmon, *Oncorhynchus tschawytscha,* in fresh water only had 0–4.5 mg.% trimethylamine oxide in muscle tissue, whereas muscle tissue in the same species taken from the sea contained 50–60 mg.%. However, significant amounts of trimethylamine oxide have been reported in muscle tissue of freshwater fishes (alewife, *Alosa,* 185 mg.%; burbot, *Lota,* 116 mg.%; trout, *Salvelinus,* and salmon, *Salmo,* 0–83 mg.%). In marine fishes the muscle tissue of the hagfish (cyclostome) has been found to contain 340 mg.% trimethylamine oxide; elasmobranchs, 250–1430 mg.%; teleosts, 25–460 mg.% (Shewan, 1951).

Ramsay (1952) has described osmotic regulation in animals as a process of *evasion* or of *correction.* In fishes, the marine elasmobranchs exemplify osmotic regulation by evasion since they have evaded the issue by falling in line with the external environment. The hagfishes (cyclostomes) have also responded in this manner, but lampreys (cyclostomes), freshwater elasmobranchs and all teleosts have employed the method of correction. They do work in order to maintain a body fluid concentration that is very different from that of the surrounding water (Fig. 4).

Knowledge of the physiology of excretion and osmoregulation in fishes has increased tremendously during the last 25 years, but there are still several aspects of the picture which should be clarified:

(1) A more complete description of excretion and osmotic regulation in the cyclostomes, and in aglomerular fishes in fresh water (e.g. *Microphis boaja*) would do much to complete the picture, and might lead to new interpretations of structure and function.

(2) Further study of nitrogen metabolism is needed with special reference to the place of ammonia and trimethylamine oxide in the physiology of fishes.

(3) The whole problem of chloride secreting cells might be reconsidered. In spite of the excellent work which has been done to prove the presence and function of these cells in the gills and head region, there are some investigators who feel that the amount of osmotic work

required of the gills of teleosts cannot be done by comparatively few secretory cells. Further histophysiological investigation of the cells of the gills is required not only in teleosts, but in marine cyclostomes and elasmobranchs as well, for a comparison of these latter two "evasion" groups with the "correction" teleosts should help to resolve the problem.

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Chapter V

THE SKIN AND SCALES

John Van Oosten

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I. THE SKIN

The skin may be looked upon as a boundary tissue that sets the individual apart as a separate entity among other organisms and in the environment. The skin with its derivatives and accessory structures forms the external covering of the fish's body through which most of the contacts with the outer world are made. The skin also maintains a continuity with the mucous membrane of the mouth and other macroscopic surface orifices of the body. It performs many important functions in fishes chiefly of a protective nature. Its derivatives play an important part in the metabolism of the body.

A. Structure

The skin of all fishes, like that of any other vertebrate, consists of two basic layers: an outer, the *epidermis,* and an inner, the *dermis* (also called *corium*) (Fig. 1). These layers differ markedly not only in position but also in origin, structure, character, and function.

The epidermis is derived directly from the ectoderm of the embryo. In the lower animal forms it retains the simple structure that consists of a single layer of cells (*simple epithelium*). In the cyclostomes and higher vertebrates, the epidermis is composed of many layers of cells (*stratified*

epithelium). The number of eell layers varies not only with the species but also with the different regions of the body and with the age of the fish. For example, in the smelt (*Osmerus*) the epidermis is thickest on the head and baek where it consists of twelve to fifteen layers, thinner on the sides of the body, and thinnest on the fins where the number of layers is only four or five.

The epithelial eells are held together by an intercellular viseous cement

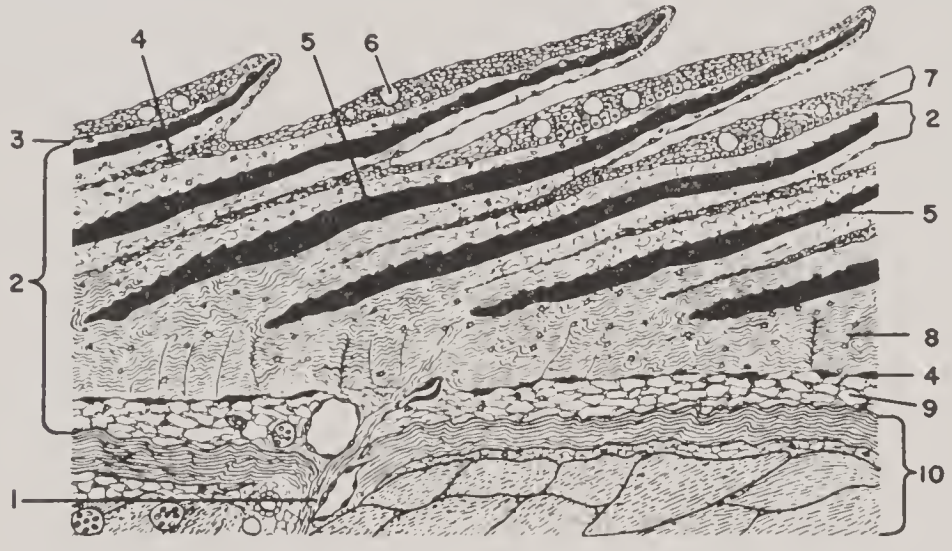


Fig. 1. Skin of yellow perch, *Perca flavescens;* 1, septum; 2, dermis; 3, basal membrane; 4, pigment cells; 5, scales; 6, mucous cell; 7, epidermis; 8, dense fibrous tissue; 9, subcutis; 10, muscle. (Courtesy General Biological Supply House, Inc., Chicago, Illinois.)

or matrix. The lowest layer, *stratum germinativum,* constructed of columnar cells, is the generating layer that gives rise to new cells. Reproduction in this layer takes place by mitotic division. One daughter cell replaees the mother eell while the other pushes upward until it reaches the surfaee where it replaees a cell that has been lost by wear or injury. During its movement to the surfaee, the cell undergoes a chemical change and gradually takes on a flattened or squamous shape. At the surface it finally dies, and is sloughed off. This desquamation is a continuous process. Nourishment for the growth and reproduction of the basal eells is obtained from the underlying vascular system of the dermis. Nutritive substanees reach the epithelial eells by diffusion through the intercellular eement.

The terrestrial vertebrates have a surface covering, the *stratum corneum,* that is made up of dead, horny cells containing keratin. In the cyclostomes, the epidermis secretes a thin film of noncellular dead *cuticle* that covers the body. In the other fishes, however, no such cornification or specialization occurs except in the pearl organs (p. 234). The

entire epidermis is alive except for the superficial layer of dead cells that have run their course and are continually being replaced.

The dermis is derived from the embryonic mesenchyme of mesodermal origin. It is composed of a fibroclastic connective tissue with relatively few cells. In the cyclostomes it forms a compact layer. In the fishes in general the dermis consists of a relatively thin upper layer of loose tissue, the *stratum vasculare* or *spongiosum*, and a thick dense lower one, the *stratum compactum*. Loosely organized connective tissue, the *subcutis*, binds the dermis to the underlying muscles. In adult fishes the dermis is usually much thicker than the epidermis to which it lends support, although considerable variation exists in the relative depths of the two sections. In young carp, for example, the epidermis is composed of two epithelial layers and the dermis of a simple compact connective tissue layer. At first the former increases in thickness faster than the latter but in the second summer the reverse takes place and the dermis then separates into two layers. The newly formed loose connective tissue first appears on the ventral side when the individual is about 10 cm. long and in the dorsal region when it reaches a length of 20 cm.

The thickness of the integument depends on the number of layers of cells and the compactness of the component layers, especially in the dermis. It also varies with the species. In many scaleless varieties, such as the North American catfishes (Ameiuridae), the skin is so tough and leathery that it can be stripped off like the hide of a mammal. In other species with scales (elasmobranchs, salmon, cod, halibut, etc.) the integument is heavy enough to be utilized in the manufacture of leather. An unusual situation is found in the ocean sunfish (*Mola*) where the rough skin is reinforced underneath by a hard cartilaginous layer which may be as much as two or three inches thick. And in the sea snails (*Liparis*) the skin may be separated from the body by a layer of translucent jelly-like substance as much as one inch in thickness.

The trunk and tail of the electric catfish (*Malapterurus*) are also enveloped in a sheath of gelatinous material that lies between the skin and muscles, but here this layer serves as the locale of an irregularly scattered number of electric plates. It may be noted that these plates are derived from modified cells of the epidermis and not from the muscular system as are all other types of electric organs in fishes (see Vol. II, Chap. V). These organs are employed to repel attacks and to stun or kill prey.

B. Chemical Composition

Comparatively little information is available on the chemical composition of fish skin since most analyses have been concerned with the

nutritive values of either the flesh alone or flesh and skin combined and many have involved canned, salted, and dried fish prepared for human consumption (see Chap. X).

In one study of the skin of three freshly caught ling cod (*Ophiodon*) that averaged 6.7 pounds in weight, the skin constituted 6.4% of this average weight. In an 11.6-pound eel (*Anguilla*), it contributed 10.7%. Approximate analysis of the skin of the ling cod gave the following mean percentages: moisture 69.6, protein 26.9, ash 2.5, fat 0.7; on a moisture-free basis, the percentages were: protein 88.5, ash 8.2, fat 2.3. Comparable values for the flesh (muscle) were: 79.2, 18.1, 1.2, 0.7% wet weight and 87.0, 5.8, 3.4% dry weight. The skin had proportionately a smaller amount of water than the flesh; the ash content was higher in the skin but the amount of protein was practically the same in both. Collagen, the mother substance of gelatin, is one of the proteins found in the skin. This constituent gives the skin commercial value in the manufacture of fish glue.

A study of the principal mineral constituents of the ash of the skin of canned coho salmon (*Oncorhynchus*) gives more detailed information as follows:

	%		%
CaO	13.51	P_2O_5	33.31
MgO	2.40	Cl	21.22
Na_2O	9.18		
K_2O	17.15	Total	96.77

Phosphorus, potassium, and calcium constituted the chief minerals of the skin of these fish. However, considerable variation occurs in the chemical composition of the flesh not only among species but also among individuals of the same species and it is probable that this holds true also for the skin.

Other studies of the integument involved isolated analyses for specific elements including nitrogen, sulfur, magnesium, zinc, copper, and iodine. In the eel (*Anguilla*), the percentage of nitrogen (4.4% of living matter) was greater in the skin than in any other organ or the body as a whole (2.56%). In this same species, sulfur comprised 0.1796% of living matter and 0.4189% of the dry weight of the skin; zinc was also present. The concentration of magnesium was about the same (0.032 and 0.035 mg. per 100 g. living matter) in the integument of *Centrophorus* and *Lophius* but greater in that of *Pristiurus* (0.125 mg.). An analysis for zinc in *Lutjanus* (*Lutianus*) and *Ailurichthys* (*Bagre*) gave the quantities 1.06 and 1.22 mg. per 100 g. of living matter, respectively. Copper

comprised 0.23 mg. per 100 g. of dry matter in the integument of *Clupea*. Some 20 iodine determinations have been made for the skin of at least 15 species of fishes. In 8 species the values expressed in milligrams per 100 g. of living matter varied from 0.016 to 0.040; in 3 species they ranged from 0.068 to 0.085; and in 2 they varied from 0.151 to 0.157. The highest concentrations were found in the cod. In *Gadus aeglefinus* the 2 determinations were nearly the same (0.31 and 0.366), in *Gadus morrhua* (= *callarias*) the 3 values were 0.550, 2.05, and 2.66.

That the chemical constitution of the skin varies with the individual fishes even though they are closely related is also evidenced by experimental transplantations. Skin grafting has been carried out successfully in the carp, *Cyprinus*, and other species but only when the transplantation was made between different sites on the same individual (autoplastic). Transplants from one individual to another of the same species (homoioplastic) or from one species to another (heteroplastic) are not usually successful in fishes (see also Vol. II, Chap. X).

C. Functions

Interspersed with the cells and in the connective tissue of the skin of fishes are a number of dermal derivatives or accessory structures that extend greatly the function of the body covering. The most common and widely distributed over the surface of the body is the simple epidermal *mucous cell* (Fig. 1) that produces *mucin*, a glycoprotein, which with water forms *mucus*, a thick, slimy, lubricating secretion. In certain fishes accumulated mucus often distends and transforms the cell into a flask-shaped *goblet cell*. Pear-shaped granular cells may also be present. The different types of mucous cells may be distinguished by their coloring, refraction, and chemical composition, although recognition is sometimes difficult.

Mucous cells are derived from the basal layer of the epidermis. When one of these cells reaches the surface, it forms a lumen through which the contents are released. Secretion may be gradual or abrupt. When abrupt the cell collapses and may then fill up again. Other cells discharge their mucus and disintegrate. Mucous cells ultimately die and are shed. A modified type of mucous gland is the thread cell of hagfishes (*Myxine*). This secretes a spirally coiled mucous thread which can be shot out and unwound for a considerable distance.

Mucous cells vary in number, kind, and size with the different species. The whitefish, for example, secretes a considerable amount of slime when handled; in comparison the yellow perch produces a small quantity and hagfishes copious amounts. Because such naked or sparsely scaled individuals as the lampreys, hagfishes, eels and certain lungfishes are much

more richly provided with mucous cells in respect to both numbers and kind than the well-scaled teleosts, some investigators have concluded that in general there is an inverse relationship between squamation and the abundance and types of mucous cells. When scalation is poorly developed or entirely lacking, its protective function is assumed by the increased number of the three varieties of mucous cells. Many heavily scaled fishes possess only the simple mucilaginous cells, some having only two layers, as in the roach (*Rutilus*).

Chemical analyses have indicated that fish mucus is largely albuminous in character. Sulfur and phosphorus were identified in the *Myxine* mucus. An incomplete analysis of the organic constituents of slime from an eel revealed that 12% was a dry substance whereof 9% consisted of albumen and the remainder of a lipoid mixture as indicated by the occurrence of the following: cholesterin, diaminophosphatides, a lecithin form of phosphatide, and a betaine-like base.

The percentage composition of the protective covering containing mucus secreted by the skin of *Protopterus* (p. 214) was reported as follows:

	%		%
SiO_2	35.838	P_2O_5	4.107
CaO	36.127	SO_3	3.614
CO_2	2.157	Fe_2O_3	9.286
Na_2O	1.415	Al_2O_3	5.491
K_2O	0.836		

Some 13.23% of the ashless matter was nitrogen.

The slippery mucus serves several purposes. It reduces body friction in the water and thus enables the fish to move at greater speed with less expenditure of energy. The slimy coating protects the living body from deleterious attack by parasites, fungus, bacteria, and other small organisms. Part of this protection is obtained by a constant secretion that tends to prevent plant and animal parasites from settling or colonizing on the skin. It is well known that removal of large patches of slime from the body surface may be fatal to the fish, especially when kept in confinement. Death may then result from an infestation of fungus or bacteria or from an interference in the normal processes of osmosis. The skin also serves in a similar protective capacity.

Mucus also retards decomposition in fishes caught for the market; most commercial fishermen appreciate this and never intentionally remove slime from their catch. Mucus, however, does not form a permanent protective coating in dead fish for after a certain length of

time the bacteria use up the nitrogen of the slime upon which they grow and then proceed to thrive on the skin. Those organisms with digesting ability may even penetrate the skin and infect the flesh.

Mucus has other protective qualities. In *Lepidosiren* (lungfish), *Pisodonophis boro* (eel) and other species, the mucus possesses a remarkable power of coagulating and precipitating mud held in suspension in water. A drop or two of mucus from *P. boro* precipitated the suspensoids in a half a pint of muddy river water in about 20 to 30 seconds. This behavior of mucus is similar to that of commercial fertilizers, manure, sludge, and other organic matter that have been used to clear turbid waters in ponds. This precipitation is the result of the neutralization of the negative charges of electricity in the suspended colloidal clay particles by the ionization of an acid or other agent that releases positive ions. Perhaps a similar chemical reaction takes place between mucus and clay.

The precipitative action of mucus may have some survival value not only in fishes that inhabit perpetually turbid waters but also in those clearwater species that are subjected for some length of time to very muddy waters resulting from heavy floods and severe storms. Clearwater fishes have been known to suffocate in extremely muddy waters (see Vol. II, Chap. IX). The precipitation of mud in immediate contact with the body, particularly the gills, would improve the chances of an individual to survive and to obtain with much less effort a continuous supply of oxygen for respiration.

In addition to this precipitative action, mucus has a lubricating effect on suspended particles which it washes away as they lodge on such delicate tissues as the gills or on other exposed parts of the body. It is well known that irritability resulting from a mechanical stimulation causes the mucous glands to increase their secretion. Consequently, healthy fishes normally can move without harm through very muddy water or water that contains considerable quantities of suspensoids other than soil. However, when chemically polluted waters reduce sufficiently the flow of mucus or the pollutants combine with the mucus, forming an insoluble compound in large quantities, the gills become clogged with deposits, and the fish dies of suffocation. The heavy metal salts such as lead, zinc, and copper that are commonly found in polluted fresh waters coagulate with the fish's slime, producing a hard film impermeable to gases.

Mucus may be of some aid to the skin in regulating whatever osmotic action takes place at the surface. The body fluids of fishes normally are less salty than sea water and more salty than fresh water (see Chap. IV). Water tends to pass into the body and salts diffuse outward in

freshwater fishes whereas in saltwater species the reverse takes place; water tends to move out and salts tend to enter the body. A thick mucous layer may very well retard these processes to some extent and thus help maintain the proper concentration of the salts in the body of the fish. However, experiments have indicated that diffusion of ions and sugars takes place almost as rapidly through the mucus of the eel (*Anguilla*) as through water, although one investigator found that the removal of mucus from a portion of the skin of an eel from fresh water actually rendered it vulnerable to the effects of salt water. In the stickleback (*Gasterosteus*) the mucous covering did not prevent an exchange of water but it did impede the passage of chloride. Young migrating eels or "elvers" can adapt themselves to abrupt changes in salinity only when their coat of mucus remains intact. It is also of interest to note here that the king or quinnat salmon (*Oncorhynchus*), when it returns to fresh water to spawn, immediately increases its production of slime, and the skin thickens considerably by the time the fish reaches the spawning ground.

The skin definitely plays a part in the osmoregulation of fishes although considerable variations exist in its permeability. The integument of cyclostomes is considered to be permeable to water and to some degree to salts. In elasmobranchs the permeability of the skin is low because its blood supply is largely confined to the impermeable denticles (p. 223) that cover the body. The skin of the bony fishes is generally permeable to water but in various degrees and it is slightly or not at all permeable to organic substances and ions. Experiments have indicated that small quantities of nitrogenous waste products are excreted through the skin of goldfish (*Carassius*) and carp (*Cyprinus*) and that chlorides, also in small amounts, are lost through the skin of trout (*Salmo*). Permeability is low in the euryhaline fishes and lower in scaly fishes than in naked forms. Experiments have shown direct losses of salts through the damaged skin of freshwater fishes and an increased loss of water and a diffusion of salts in similarly injured marine species.

Mucus is used for a unique protective function in the African lungfish, *Protopterus*. With the approach of the dry season these fishes dig a burrow in the mud in the lower portion of which they encase themselves in a hard cocoon of mucus mixed with silt. During this dormant period the skin is kept moist also by mucus. With the advent of the rainy season the individual emerges and resumes its active life.

Mucus also serves a special function in the construction of nests by male sticklebacks (Gasterosteidae) during the breeding season. These nests, similar in appearance to those of orioles, are built out of sticks, stalks of plants, or any other similar materials at hand, which are woven

together and held in place by threads of a sticky substance secreted by the kidneys. The male then rubs his sides against the structure, both on the outside and inside, and works the body mucus into the materials where it hardens like mortar. The fighting fish (*Betta*), the paradise fish (*Macropodus*), and other related species also employ a mucus in constructing a very unusual type of nest. The male blows bubbles of air and a sticky mucus into the water, and forms a floating mass of foam to the underside of which the eggs are stuck.

Another structure with a special function found in the skin of a number of species is the *poison gland,* also derived from the epidermis. These organs are usually associated with the spines on the fins, tail, and gill cover. The poison is produced either from glandular cells among the tissues that line or lie along the grooves or from a sac or tissues found at or near the base of a spine. Poison glands are found in certain sharks, rays, chimaeras, and in a number of bony fishes, including catfishes (Siluridae), weevers, (Trachinidae), scorpion fishes (Scorpaenidae), and toad fishes (*Opsanus*). The virulence of the venom varies considerably among the individuals of these groups, but it is probably seldom great enough to be fatal to man. The function of this poison is not too clear but, since it is associated with spines, it is probably used for offense as well as for defense.

Phosphorescent or luminous organs called *photophores* occur in the skin of a number of marine fishes (see Chap. VI, Vol. II). These structures are formed by a modification of the cells derived from the germinative layer of the epidermis.

Special structures or organs also occur in the dermis. *Chromatophores* are modified dermal cells that contain pigment (Fig. 1). The various types are distinguished by the nature and color of their pigment; they may be black (melanophores), yellow (xanthophores), red or orange (erythrophores), or white (guanophores or leucophores) (see Vol. II, Chaps. VII and VIII). There may also be present platelike crystals (guanine), the *iridocytes* or *iridophores*, which by their reflective and iridescent qualities can change the color effect of the pigmented chromatophores. Where pigment cells are absent, the massed iridocytes can give the body a dead white color, usually on the ventral side.

Body colors vary with the abundance and disposition of the iridocytes and chromatophores in the skin and underlying tissues and the dispersion of the pigment in the cells. Many fishes acting largely through sight can adapt the color, and some the color pattern, of their skin to the background, sometimes so effectively as to make the individual inconspicuous (see Vol. II, Chap. VIII). Many fishes go through a series of color changes with increasing age as part of their natural development.

Highly conspicuous derivatives of the dermal layer of the skin are the bony *scales, shields* or *plates,* and *denticles* (the enamel-like surface of denticles, however, is derived from the epidermis) that form the elements of the *dermal skeleton,* sometimes referred to as the *exoskeleton* in contrast to the *endoskeleton,* the bony structures that lie inside the body. The primary function of the dermal skeleton is protection of the body. It also supports and strengthens the underlying integument, which as a rule is much thinner and more tender than in the scaleless fishes, and it helps the body to maintain its proper form. Scales are often considered part of the skeleton rather than of the integument and they have come to form the major basis of several fields of research, so they are here given a status equal to that of the skin even though they are, together with other structures, derivatives of the skin. These external bony structures will therefore be treated later in greater detail (see pp. 219 ff.).

The skin is an important adjunct to the breathing equipment of certain fishes (see Chap. I). The eel, *Anguilla,* and the mudskipper, *Periophthalmus,* that are able to live for a considerable time out of water, can respire through the wet skin. This breathing is made possible, at least in part, by the rich supply of blood vessels in the dermis. In the mudskipper the head has an increased vascularity and it functions as a special respiratory structure together with the pharynx. This fish can remain out of water for indefinite periods. In virtually all other fishes, however, skin cannot function effectively in respiration as it does in many amphibians, although there is considerable variation among species. Carp, for example, can endure exposure to air for a much longer period than trout. This difference, however, is not due entirely to a variation in skin respiration; it is largely the result of a discrepancy in oxygen demand which is high in trout and low in carp.

Fishes are poikilothermous, that is, their body temperature varies passively with that of the environment so that it is low in cold water and rises in warm water. The numerous capillaries of the circulatory system in the dermis and especially in the gills play an important part in this regulation. When these capillaries are dilated (by increased blood flow), heat is lost; when constricted, more heat is retained. Heat elimination is relatively rapid in fishes since their bodies are not heavily insulated against heat radiation by thick layers of fat in the subcutaneous connective tissue.

As a rule, under normal conditions the temperature of a fish's body approximates that of its environment, although there are variations and exceptions. Fishes generate, although at a much lower rate than that of the warm-blooded animals (homoiotherms), a certain amount

of internal heat during muscular and metabolic activities. The fast-swimming tuna (*Thunnus*) is said to have a temperature considerably higher (6–12° C.) than that of its habitat. There are, of course, limits to the temperature adjustments which can be made by fishes and the body temperature can never go below that of the surrounding water. The differences between body and environmental temperatures have been found to vary with the species from less than 1 degree to as high as 12° C. (21.6° F.). However, several factors have been involved in these widely different results. The early investigators usually employed ordinary thermometers while later research workers have generally used the more exact thermoelectric method. The size of the specimen examined has not always been taken into consideration. As the individual grows the ratio of its surface area to its body mass declines so that the heat-losing capacity of the surface does not keep up with the heat-producing capacity of the mass. A large individual can thus generate and retain relatively more heat than a small fish and, therefore, may have a greater heat gradient within its body. The temperature of a large specimen would then vary with the locale of the recording thermometer, the highest being inside. The thickness of the skin and other tissues and their thermal conductivity also affect the capacity of the body to conduct heat. Stimulation or excessive activity such as may result from handling also increases the body heat above its normal.

As has already been indicated, the skin is the locus of many special or modified structures. Those that were discussed briefly in this section included mucous cells, poison glands, photophores, chromatophores, iridocytes, scales, plates, and denticles. Blood and lymph vessels, nerves, and muscle fibers are located in the dermis, but the peripheral terminations of many nerve fibers, the receptors, are found in the epidermis. These receptors include the neuromasts of the lateral line, taste buds, pit organs, and free nerve endings. The numerous sensory organs (exteroceptors) scattered over the body of the fish make the skin one of the most important receptors of physical and chemical stimuli from the outside (see Vol. II, Chap. II). This function is of the greatest survival value to the individual since the skin provides the principal means of contact with the surrounding environment.

Another major function of the skin is the repair of surface wounds. Injuries in which the superficial layers of the epithelium are lost are readily repaired by the cells from the margin of the wound. Immediately following an injury they close the wound by secreting a mass of mucus which is mixed with large numbers of lymphocytes. On this slippery surface the cells advancing from the margin glide into the damaged area and after a day or so form a thin protective epithelial layer by cell

proliferation. Within a few days the enclosed dead cells collect at the surface of the wound where they create a turbid or cloudy appearance. A covering epithelial layer forms much more rapidly in fishes than in warm-blooded animals but after the wound is covered further recovery in fishes is extremely slow.

Coagulated blood plays only a small role in the healing of injuries in fishes because of the relatively small amount of this fluid available and its low pressure (see Chap. II). It has also been reported that superficial wounds are closed and temporarily protected by a coagulating albuminous serum presumably secreted by lymphatic cells.

A deep injury that penetrates the musculature and destroys part of the basal layer is not as readily healed. In the carp such a cut fills up rapidly with a fairly clear transudate and a film-forming fibrin mixed with blood corpuscles. The basal layer then penetrates the wounded area and shuts off the newly formed epithelium underneath. The subcutis area fills up with a granulated tissue formed from the connective tissue. A return to normal requires months.

The time required to close or heal a wound varies with the species, individual, age of the fish, extent of the wound (size and depth), temperature, and many other external and internal factors. With experimental cuts that penetrated the muscles of the pond loach (*Misgurnus*) a slight advance of the epithelium over the wound occurred within an hour. After 6 hours, fibrin covered the wound and after 12 hours, the epidermis had completely bridged the cut. After nearly 3 months, conditions approached normal except for the lower layer of the skin and the underlying musculature. In the common killifish (*Fundulus*), however, injured surfaces healed and scales began to "regenerate" within 10 days.

Another possible function of the skin is the absorption of food. Goldfish kept in water previously inhabited by other goldfish (conditioned water) grew much faster than those held in unconditioned water on the same feeding schedule (see also Chap. IX). The increased growth was attributed in part to growth-promoting chemicals secreted from the skin of the first inhabitants, but the chief cause was the absorption through the skin of the dissolved minute particles of regurgitated food left by these individuals. Similar results were obtained by dissolving nutrients in the water which enabled goldfish to survive twice as long without food as when kept in untreated tap water. Greater absorption through the skin may account for the greater growth rate of the mirror and leather carp as compared with the scaled form under identical conditions.

Certain substances secreted by the skin and perceived by smell may affect the behavior of fishes (see Vol. II, Chap. II: Part III). These secre-

tions may have an attractant stimulus and assist the individuals of a species in shoaling or, as demonstrated in *Phoxinus phoxinus,* injured skin may give off a substance that evokes an alarm response in a school of fishes.

It is now evident that the integument, considered as a whole with its accessory structures, serves many purposes in fishes. The most obvious role is protection. The skin covers the body and protects the deeper tissues against injuries and infections. The dermal skeleton performs the same function. The stout erectile spines of such species as the porcupine fishes and the bony encasements or shields of other species ward off attacks by predaceous enemies. The adaptive blending of the color pattern of the skin with the environmental background also protects the individual from predators. The skin and its mucus prevent the invasion of the body by parasites, fungus, bacteria, and other microorganisms. They also perform a major function in repairing surface wounds and aid the osmotic processes that regulate the water content and salt concentrations in the body fluids. Mucus by its lubricating and precipitating action on suspensoids may give relief to those fishes that experience a suffocating strain in highly turbid or polluted waters. Thermal adjustment to the environment is made in part through the skin. It may also serve as a respiratory organ. It has the power to absorb food and to secrete substances that stimulate behavior patterns. It is a receptor of stimuli. It is the seat of many organs with a special function. It is the principal organ of contact with the outer world.

II. SCALES

In most fishes much of the connective tissue of the dermis is replaced by the dermal skeleton in the form of scales (Fig. 1). Various types of these structures have been described, some of which no longer exist in living fishes. They have been classified as *cosmoid, ganoid* or *rhomboid, placoid, cycloid,* and *ctenoid.* Many of the earliest fish-like fossil vertebrates, the ostracoderms and placoderms of the Silurian and Devonian eras, were armored in various degrees with plates or shields and scales of different shapes that consisted typically of three layers or zones. The inner layer was constructed of compact lamellar bone (*isopedine*), the middle of spongy vascular bone perforated with anastomosing canals used by blood vessels, and the outer of dentinal material. The surface of the plates and scales was sometimes covered with ridges or tubercles of dentine. These tubercles, also forming separately a body covering in some species, resembled the modern elasmobranch denticles in structure (p. 223). It is likewise of interest to note that one group, the acanthod-

ians, possessed scales that were structurally nearly the same as the ganoid scales (p. 223) of the higher bony fishes of the present era.

A. Cosmoid Scale

The two principal structural types of scale found in the early bony

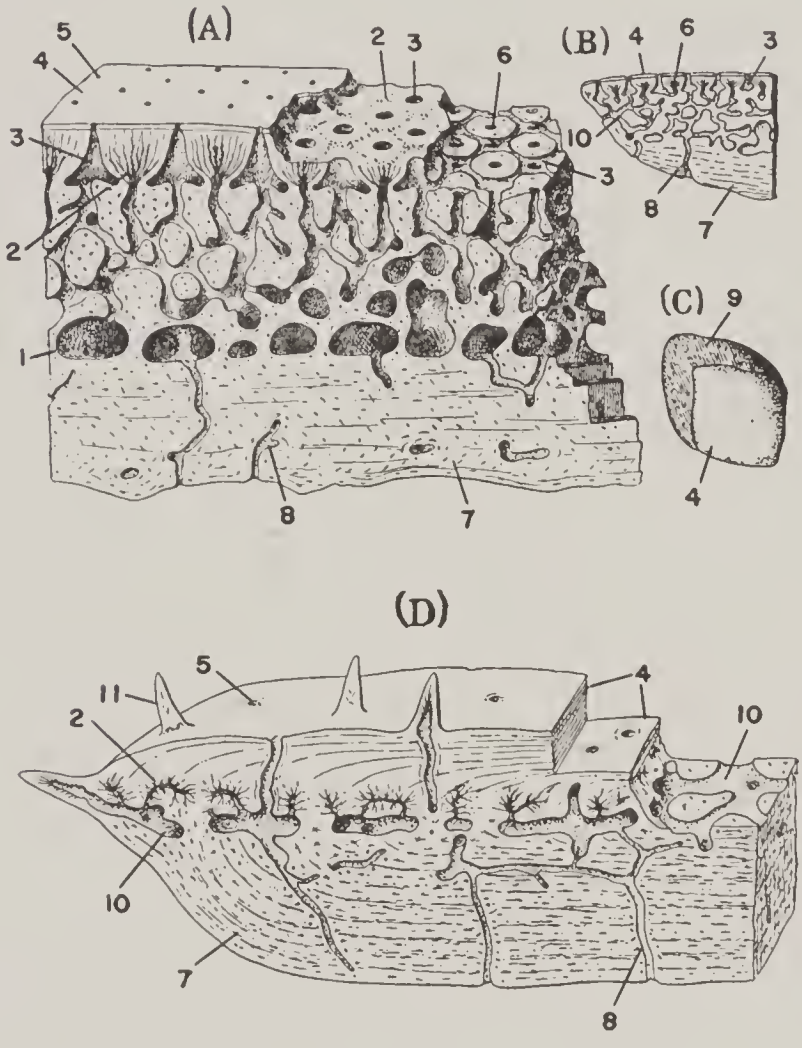


FIG. 2. A: transverse section, B: section through posterior end, C: surface view of a cosmoid scale. D: transverse section of a true ganoid scale of *Polypterus bichir*. 1, large vascular cavity; 2, canaliculi of cosmine layer; 3, chamber of cosmine layer; 4, vitrodentine (cosmoid) or ganoine (ganoid) surface layer; 5, opening of chamber (cosmoid) or vertical canal (ganoid); 6, pulp cavity from which canaliculi radiate; 7, lamellar bony layer (isopedine); 8, vertical canal; 9, covered anterior region; 10, irregular (cosmoid) or horizontal (ganoid) vascular canals; 11, superficial denticle. (From E. S. Goodrich; courtesy A. and C. Black Ltd., London.)

fishes are the cosmoid and ganoid. The cosmoid scale (Fig. 2 A, B, C), occurring only in the fossil crossopterygians and lungfishes, is similar to the placoderm scale in structure except that superimposed on the two basal layers of bone is a stratum composed of a noncellular, dentine-like substance called *cosmine*, which in turn is covered with a thin layer

of enamel-like *vitrodentine*. The cosmine layer contains numerous fine radiating dentinal tubules, pulp cavities, and vascular chambers that lead to openings on the surface. Cosmoid scales in general grew by the addition of new isopedine layers below and the deposition of materials around the periphery of the upper strata, enclosing the newly formed chambers and pulp cavities. Cosmoid scales were either rhombic or cycloid in shape (p. 225); they were either articulated by means of a peg and socket joint with adjacent scales or isolated and imbricated.

The cosmoid scale is no longer found in living fishes. The three genera of living lungfishes (Dipnoi) found in Africa, South America, and Australia have lost completely the cosmoid scales of their ancestors; they now possess thin, bony cycloid scales devoid of cosmine and ganoine. The recently discovered (1938 and later years) living crossopterygians (*Latimeria*) have the typical imbricated, coelacanthid scale of their ancestors. These scales were either cycloid or, as in *Latimeria,* they resemble the ctenoid scale in appearance and structure except that the exposed surface is studded with fixed placoid denticles.

B. Ganoid Scale

The true ganoid scale (Fig. 2D), found abundantly in the primitive actinopterygian or ray-finned fishes, particularly the palaeoniscoids, differed from the cosmoid scale mainly in having the thin surface covering replaced by many layers of a hard, glistening, enamel-like, cell-less substance called *ganoine*, a material composed almost entirely of inorganic bone salts. This variation from the cosmoid scale was the result of a difference in the method of growth. The ganoid scale increased its size by the addition of new layers to both the lower and upper surfaces.

Immediately below the ganoine cover lies the dentinal layer with its minute branching canaliculi and vertical and horizontal network of vascular canals. It is at the periphery of this layer that the growth of the scale begins. The lowermost and thickest zone consists of lamellar bone that also contains canals. These laminae bend upwards at the periphery and join the corresponding elements of the middle layer. These scales are usually rhomboidal in shape but may also be cycloid and deeply imbricated. Their articulating peg-and-socket joints consist of bone. Some scales have small, sharp denticles fixed to the upper surface.

The true ganoid scale occurs in living fishes but in somewhat modified form and to a limited extent. It has persisted in different degrees only in the most primitive surviving representatives (Chondrostei) of the actinopterygians. The scales of the bichirs (Polypteridae) are the least modified (Fig. 2D). These fishes are completely invested with the thick,

three-layered, articulated scales, but the sturgeons (Acipenseridae) and paddlefishes (Polyodontidae) have lost the cosmine and ganoine layers, although remnants of the primitive rhomboid scales remain on the upper lobe of the caudal fin and the larger scutes of the sturgeons still possess an elaborately canalized surface layer. The body of the paddlefish is

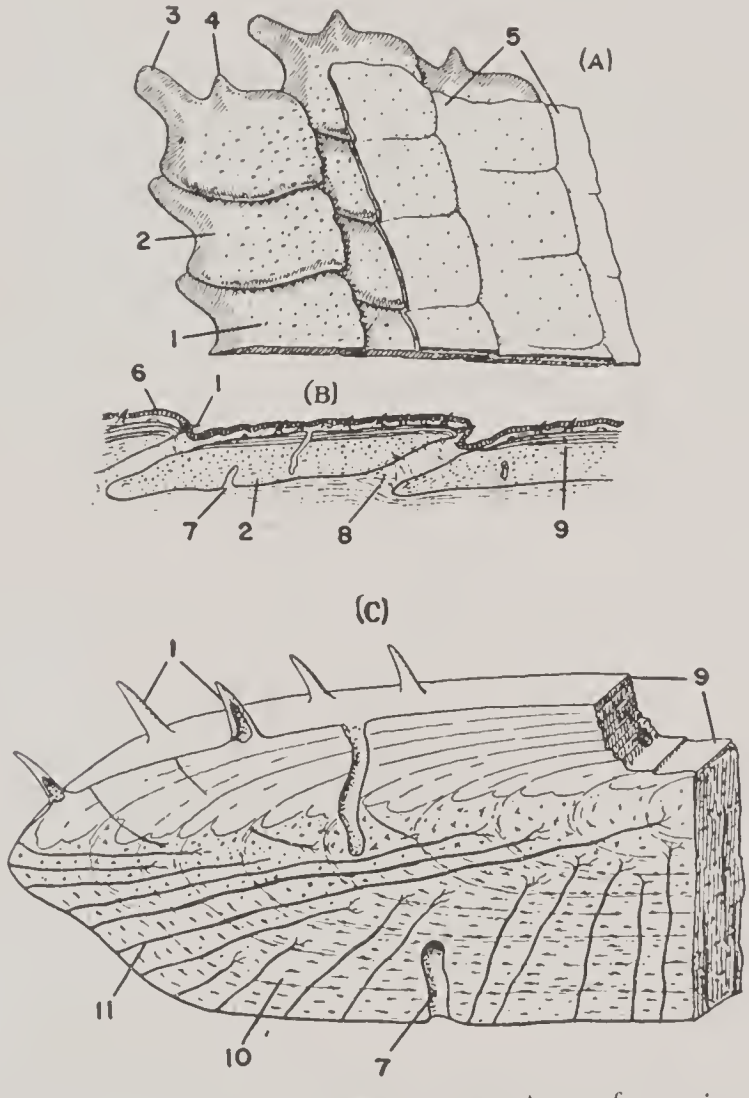


FIG. 3. Scales of the gar, *Lepisosteus osseus*. A: surface view of trunk scales from which skin and connective tissue have been removed on the left side; B: longitudinal section through skin and scales; C: transverse section of scale. 1, denticle; 2, scale; 3, anterior articulating process; 4, dorsal articulating process; 5, skin covering scales; 6, epidermis; 7, vascular canal; 8, connective tissue of dermis; 9, ganoine layer; 10, lamellar bony layer (isopedine); 11, tubules with branching inner ends. (From E. S. Goodrich; courtesy A. and C. Black Ltd., London.)

actually naked except for isolated vestigial denticles embedded in the skin. The sturgeons, however, carry five longitudinal rows of spiny (in the young), bony, articulated scutes or bucklers on their trunk, interspersed with oblique rows of small, bony denticles each of which consists of an embedded rhomboidal or diamond-shaped basal plate with

one or more protruding spines. In all other living bony fishes the cosmine layer has disappeared and the ganoine has persisted only in the gar pikes (*Lepisosteus*). These changes have decreased considerably the thickness and rigidity of the scales.

In gars (Fig. 3) the scale is composed of thick ganoine layers superimposed on strata of lamellated bone with which they merge at the periphery. In the absence of the cosmoid system of canals and cavities, the gar scale is traversed from top to bottom by a few simple, large, and usually unbranched canals that contain mesodermal cells and blood vessels. The canals serve to connect the dermis below and above the scale. In addition there are numerous slender tubules of various lengths, about 3 μ in diameter, the inner extremities of which are branched. These tubules run inward mostly from the lower and lateral surfaces more or less at right angles to the bony laminae. Each tubule contains a protoplasmic process sent out by a cell on the surface. The function of the tubular system is probably trophic. The gar scale grows by the deposition of completely new layers to both surfaces, each successive layer being larger than the preceding one. The ganoine layer varies in thickness up to about 100 μ (compared with about 50 μ in *Polypterus*) and bears numerous placoid denticles that have become fused with it. The roughly rhomboidal, slightly overlapping plate-like scales articulate with one another by peg-and-socket joints at the margins as in the bichirs and are also bound together by connective tissue. Only the exposed portion of these scales is covered with ganoine, again as in a bichir scale. The scales of the other living holostean, the bowfin (*Amia*), however, do not possess a ganoine covering; they are, even histologically, typical imbricating cycloid scales.

C. Placoid Scale

With rare exceptions, placoid scales are restricted to the sharks, rays, and chimaeras (Chondrichthyes). In most sharks they cover the entire body in a series of oblique rows. In the rays they are scattered irregularly and in some species are entirely lacking. In the chimaeras scales have almost disappeared; they occur only in certain highly localized areas on the body.

A typical placoid scale (Fig. 4) consists of a flattened, rectangular *basal plate* embedded in the upper layer of the dermis, and a protruding *spine* that projects backward on the surface of the body. The placoid scale resembles a tooth in structure. The spine consists of a cap or cone of *dentine* that encloses a *pulp cavity* from which radiate numerous branching canaliculi called *dentinal tubules*. The cavity is often subdivided into larger branching canals which also give off these tubules.

The basal plate is perforated by one or more openings through which the pulp maintains contact with the superficial layer of the dermis and through which the blood vessels, nerves, and lymph channels enter the cavity. The spine is covered with a layer of hard, transparent, enamel-like *vitrodentine*. Placoid scales vary in shape; they may be conical, knob-like, or flat. They may fit close together or be set apart. They do not overlap except where they protect the lateral line canal. Placoid scales do not continue to grow indefinitely. When old, worn out, or lost, they may be replaced.

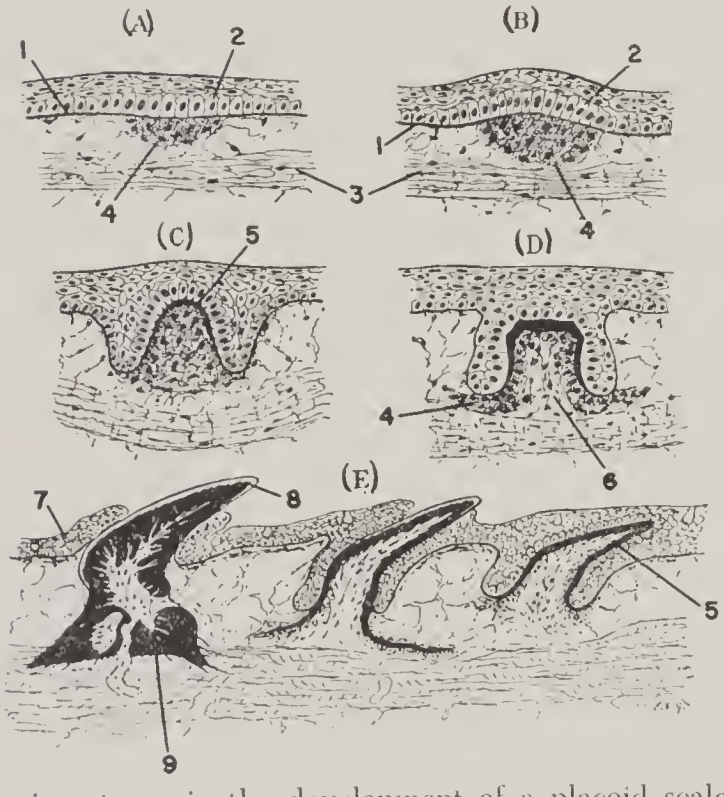


FIG. 4. Successive stages in the development of a placoid scale from the shark, *Scyliorhinus canicula*. A–D: transverse sections; E: longitudinal section. 1, basal membrane; 2, modified epidermal cells (ameloblasts); 3, connective tissue of deep layer of dermis; 4, formation of papilla and layer of odontoblasts; 5, dentine (black); 6, pulp cavity; 7, epidermis; 8, "enamel" or vitrodentine (white); 9, basal plate. (From E. S. Goodrich; courtesy A. and C. Black Ltd., London.)

The placoid scale arises as a papilla (Fig. 4) formed by the multiplication of the fibroblasts in the upper layer of the dermis. This papilla, as it grows, pushes upward into the stratum germinativum of the epidermis. Dermal fibroblasts accumulate on the surface of the papilla and form a distinct layer of cells called *odontoblasts*. A dermal fibrous matrix appears between the stratum of these cells and the basal layer of the epidermis. The odontoblasts impregnate this matrix with calcium salts forming the first layer of dentine that covers the papilla. Odontoblasts, unlike the bone-producing cells, never form part of the calcified

tissue; they continue to lie on the inner surface of the dentine adjacent to the pulp. However, ultimately, as the formation of dentine continues, the deep-lying cells do penetrate the dentine by means of cytoplasmic processes that extend into the dentinal tubules. These tubules are formed by the earlier odontoblasts that send out protoplasmic processes around which the dentine is deposited. As the dentinal layer thickens, the core of the papilla is reduced. This central area remains uncalcified and forms the pulp. As the scale grows, its lower portion may extend inward, as it usually does, into the fibrous layer of the dermis forming the basal plate. Since the odontoblasts can move on all sides of this plate, it may increase in size in all directions.

After the scale is formed and before it erupts, the cells of the stratum germinativum that had covered the papilla are modified into *ameloblasts*. These cells then deposit a layer of cuticle on the dentine which on being impregnated with calcium salts forms the "enamel" or vitrodentine of the spine. The fully formed spine finally breaks through the epidermis, losing the covering ameloblasts during the process. It may be noted here that the placoid scale has a dual origin in contrast to the other three current types of scales that are derived entirely from the dermis.

D. Cycloid and Ctenoid Scales

The vast majority of our living bony fishes are teleosteans. Their scales have lost all trace of the cosmine and ganoine layers so that now, as a rule, they have been reduced to thin, flexible, transparent structures. When fully developed, they are either cycloid or ctenoid (see Fig. 7, p. 235). The former, the simpler of the two, is the characteristic scale of the more primitive teleosts—fishes with soft-rayed fins. The latter is the typical one of the more advanced groups—fishes with spiny-rayed fins. However, both kinds may occasionally be found in the adults of the same species as, for example, the mud dab (*Limanda*).

The two types of scales are differentiated primarily on the basis of their surface sculpture and their origin and development are fundamentally very similar. The ctenoid scale bears stiff spines (*ctenii*) in the posterior area whereas the cycloid scale has none. Sometimes these ctenii are soft and barely noticeable when they are referred to as *ciliated* spines. Other surface structures common to both types of scales are described in connection with the classification of fishes (p. 234).

It has long been accepted by all investigators that the typical teleost scale consists of two major parts, a surface layer referred to by various authors as the "bony," "hyalodentine," "sclerite" . . . layer and a deeper fibrous section variously called the "lamellar layer," "fibrillary plate," "basal plate." However, with respect to the histogenesis of scales there

is no such unanimity of views. This situation may have arisen because of the variability of the materials and techniques employed. To determine the sequence of events in any histological study is a difficult undertaking; to analyze the intricate processes involved in the origin and development of the tissues is even more complicated. It does not come within the scope of this chapter to present the controversial issues of scale development but in order to provide the basic information on the

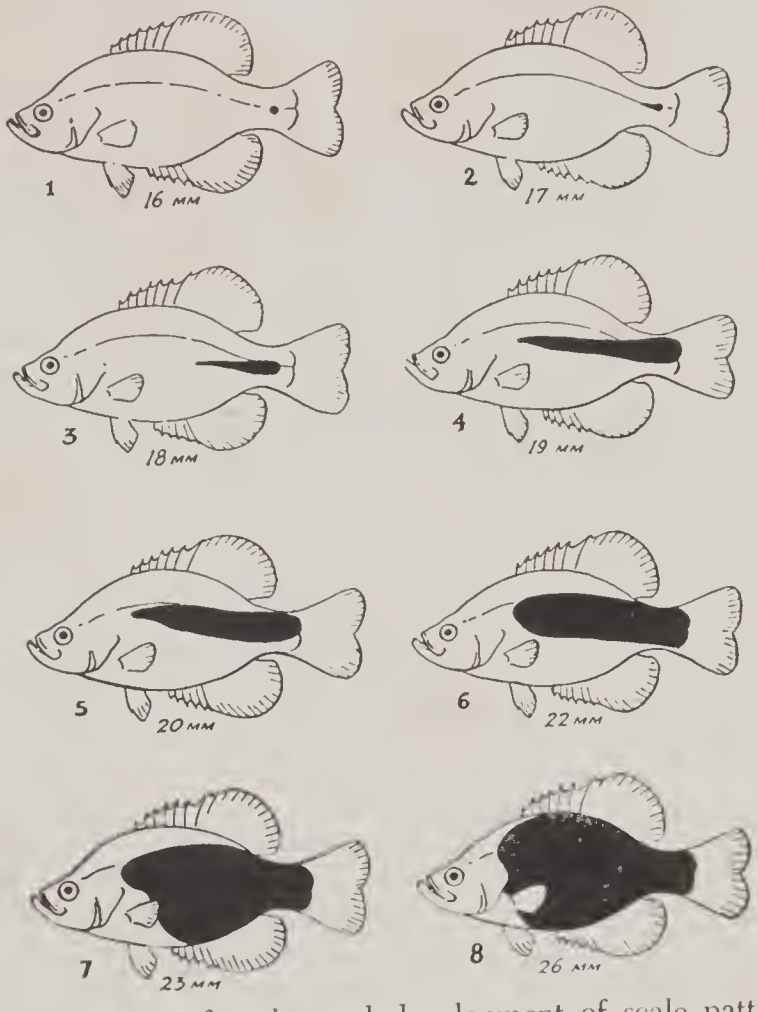


FIG. 5. Point of origin of scales and development of scale pattern of the black crappie, *Pomoxis nigromaculatus* (from H. C. Ward and E. M. Leonard, 1954).

histology and histogenesis of a teleost scale, the processes involved in the development of trout scales are presented with some modifications.

The teleost scale has its origin in a dermal papilla formed by the mitotic multiplication of the fibroblasts. In the several varieties of trout the papillae first appear on or adjacent to the lateral line in close association with the neuromasts. As a matter of fact in most teleosts the first scales to appear are those in close relation to the lateral line, although additional centers of origin of secondary importance also occur in some

species as, for example, the brook trout (*Salvelinus*), marine herring (*Clupea*), alewife (*Pomolobus*), cutthroat trout (*Salmo*), black crappie (*Pomoxis*), and smallmouth bass (*Micropterus*). The most common region of first occurrence is along the lateral line on the caudal pedumele from where the scale pattern develops as the number of rows increases and the scales spread in all directions. In trout the forward-extending rows of scales continue to arise first along the lateral line and then spread out from there, but in the Centrarchidae (Fig. 5) they make their first appearance some distance below and apparently independently of the lateral line.

In teleosts that have been investigated, scales first appear at an average

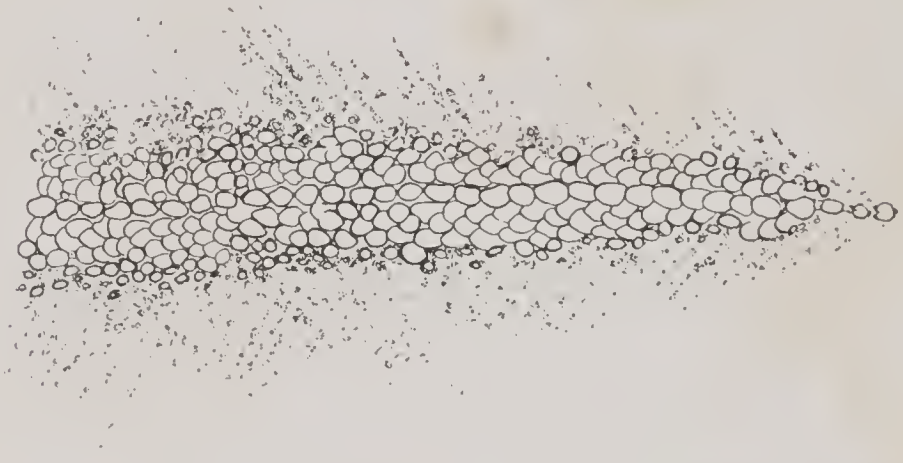


FIG. 6. Development of the scales of a brook trout, *Salvelinus fontinalis*, showing scale pockets, scale papillae, and active outgrowths of dense tissue below and above the lateral line (from P. F. Elson, 1939).

total body length that varies with the species generally from about 12 to 50 mm. Scales no doubt form at a body length less than 12 mm. in species that attain only a small size. In the eel (*Anguilla*), on the other hand, they do not appear until the fish reaches a length of about 160 to 200 mm. in the third or fourth year of life. This delayed formation is no doubt related to the late metamorphosis of the eel that occurs at a length of about 80 mm. In the trouts the individuals ranged from 25 to 32 mm. in length when the first papillae appeared.

Each of the lateral line (primary) papillae of the trout produces an active outgrowth ventrally and dorsally, a cellular process that extends outward and obliquely forward from the lateral line and between the dermis and the stratum germinativum (Fig. 6). At intervals along each outgrowth, the fibroblasts multiply and produce other (secondary) papillae. Each row of papillae ultimately becomes a row of scales. However, irregularities and branching occur in the formation of these rows of

papillae which is evident from the presence of extra intercalated scales and double scales and the discrepancies between the number of scales in the lateral line and the rows adjacent to it. Branching of the outgrowths commonly takes place in the anterior part of the body. Sometimes some of the outgrowths extend obliquely posteriorly but are checked on meeting the normal forward growing line.

Each papilla at first is solidly composed of fibroblasts but soon the center is occupied by a fluid or gelatinous intercellular substance into which the cells migrate to form a central mass. Those cells that remain at the periphery form the outer follicle that later comes to lie around the margin of the scale pocket and produces the cells that form the bony margin of the growing scale. The cell mass spreads out and forms two layers between which appears a network of fibrous material. Through the activity of these investing cells, called *osteoblasts* or *scleroblasts,* the surrounded osteoid tissue becomes impregnated with calcium salts thus initiating the formation of the bony or "hyalodentine" layer.

Subsequently the fibrillary plate appears as a thin sheet between the bony scale and the lower layer of osteoblastic cells. Later these cells disappear and the plate then lies between the scale and the underlying connective tissue. Some believe that the initial layers of the plate develop from the osteoblastic cells; others state that it is more logical to assume a single origin for the entire plate: that it arises from the connective tissue and that the initial laminae are derived from substances that pass through or between the osteoblastic cells.

As the small platelet grows, it changes from a horizontal position to an oblique angle. The posterior end pushes upward raising both layers of the skin while the anterior margin sinks deeper into the dermis gradually forming a scale pocket. Most of the trout scale lies buried in the dermal pocket. The scales of some species of fish are shallowly embedded. The oblique position exposes a small triangular portion of the posterior area so that this part is covered on both surfaces by the dermis and the epidermis. When a scale is pulled out of its pocket, the skin adheres to this posterior area whereas the deeper-lying anterior portion comes out clean. The changed position also results in the overlapping of the posterior region of one scale onto part of the anterior of another behind it, much like the shingles of a roof. The fully developed scales are usually arranged on the body in a series of diagonal, transverse rows.

Growth of the formed scale is continued, generally throughout life, by accretion at the margin of the surface layer and the deposition of thin fibrous layers below it. Because the bony deposits are made at the periphery before the fibrillary plate reaches it, at least in the trout, the

upper stratum normally extends beyond the lower during the growing period. Since the surface layer grows only by deposition of materials at the edge, it does not increase in thickness with the age of the fish nor do the early superficial features change except for wear. This latter fact makes possible the determination of age from scales. The fibrous section, however, increases in depth from the margin towards the center, since each new layer covers the entire lower surface of the scale and extends beyond the preceding one. The thickest part of the scale, therefore, is always in the center. The scale may be thought of as a greatly flattened cone.

Each sheet or lamella of the scale is constructed of fibrils that lie more or less parallel to one another but at sharp angles with those of the two adjacent lamellae. This type of structure imparts considerable firmness to the scale. The fibrillary plate is largely or entirely uncalcified and without vascular canals. As already indicated the upper layer of the scale is composed of an organic framework impregnated with inorganic salts, chiefly calcium phosphate and calcium carbonate. It contains no bone cells. The epidermis takes no part in the formation of teleost scales and hard enamel-like substances are lacking.

There are apparently variations in the method of scale formation. Recent studies indicate that calcification in the goldfish scale is not restricted to the periphery but occurs throughout the entire scale between the formed bony and fibrillary layers. The salts are probably secreted from the floor of the scale pocket and reach the inner layers through the fibrillary plate. Under this process, then, the bony layer also increases in thickness as the scale grows.

These results were obtained by means of a new staining technique. Lead acetate was injected into the thick dorsal muscles of the fish and subsequently a layer of lead salts was deposited in the scales, fin rays, vertebrae, and opercula (not otoliths) where active calcification took place. Conversion of the lead salts to the sulfide produced a dark line easily observed in cross-section. Further calcification was demonstrated by a second injection after an interval of several months. By this method it was possible to identify the area of active calcification below the bony layer and at the margin of the scale.

A recent investigation has also revealed that the scale of the eel is not constructed of a lower fibrous layer superimposed by a group of platelets but consists of a series of loculi arranged concentrically. Each loculus is formed by closely allied fibers with intercommunicating threads that extend from one surface to another. Particles of calcium salt are distributed in and between these fibers. Zones of loculi representing a year's growth are separated by a narrow band of fibrous tissue.

E. Chemical Composition

Only a few studies have been conducted on the chemical composition of fish scales. They indicate that these scales contained from 41 to 84% organic protein and up to 59% mineral residue in dry matter. The former consists mainly of collagen and ichthylepidin. The relative amounts of these two albuminoids when both are present is nearly constant in the scales of different teleost fishes, about 76% ichthylepidin and 24% collagen. However, ichthylepidin, although widely distributed in the scales of teleosts, was not observed in all species investigated. No trace of it was found in the dermal skeleton of the sunfish (*Mola*), puffer (*Sphaeroides*), gar (*Lepisosteus*) or in elasmobranchs. It is evident that the organic constitution of scales is not the same in all fishes.

An analysis of moisture-free menhaden (*Brevoortia*) scales showed 58.9% organic matter and 41.1% ash. The moisture content of the air-dried scales amounted to 20.6%, and the organic matter constituted 46.8% and ash 32.6%. It is of interest to note that the scales of an average-sized menhaden (5.5 ounces) comprised about 4.5% of the body weight while in the alewife (*Pomolobus*), this value was about 3%.

The percentage composition of the mineral residues (dry weight) varies with species, as shown below:

Species	Ash in dry matter	$Ca_3(PO_4)_2$	$Mg_3(PO_4)_2$	$CaCO_3$	CaF_2
Alosa	–	98.38	1.79	0	0.29
Sardinia	43.43	7.88	0.69	21.37	–
Cyprinus	16.23	56.74	1.68	41.57	–
Cyprinus	29.58	51.42	6.45	42.17	–
Esox	40.44	53.25	5.01	41.73	–
Cololabis	58.23	1.93	0.74	17.34	–

According to these analyses, the inorganic salts were principally calcium phosphate and calcium carbonate. Magnesium phosphate and calcium fluoride, when present, occurred in small quantities. Magnesium was also found in *Labeo* scales (8.831 mg. per 100g. dry matter), and fluorine was estimated in scales of other fishes as 0.049% wet weight, 0.060% dry weight, and 0.13–0.144% ash. Sodium carbonate and traces of sodium chloride and iron have also been reported. Sulfur constituted 0.2776% of the living matter (0.4436% dry weight) of the scales of the carp, *Cyprinus*. *Serranus* scales were analyzed for arsenic but only a small quantity was obtained (0.005 mg. per 100 g. dry matter); the body of

this species, however, contained the smallest amount of arsenic (0.005 mg.) of any of the numerous fishes investigated.

Another study gave the following figures for the percentage composition of carp and pike (*Esox*) scales:

Species	CaO	MgO	P_2O_5	CO_2
Carp	15.98	0.48	13.12	1.43
Pike	21.93	0.51	18.00	2.30

F. Variability in Squamation

Not all teleosts have cycloid or ctenoid scales. The snipefish (*Macrorhamphosus*), for example, has scales that are similar to dermal denticles with a basal plate, the spine even containing a pulp cavity. Other teleosts have a similar type of scale but with a solid spine that is either single as in the batfish (*Ogocephalus*) or branched as found in the frogfish (*Antennarius*). In triggerfishes (*Balistes*) and filefishes (*Monacanthus*) the basal plate of the scale may carry a number of spines. In some of the mailed catfishes (Loricariidae) each plate has a group of movable spines set in sockets. These spines have essentially the same structure as the placoid scale—a very rare occurrence among the teleost fishes.

Pipefishes and sea horses (Syngnathidae) possess a dermal skeleton of bony plates arranged segmentally. Such diverse varieties of teleosts as the South American mailed catfishes (Loricariidae), alligator fishes (Agonidae), and trunkfishes (Ostraeiidae) have the body more or less completely cuirassed with bony shields. The porcupine (Diodontidae) and globe fishes (Tetraodontidae) bear sharp bony spines that stand erect when the fish inflates its body. Scales are represented by embedded dermal prickles in the sculpins (Cottidae) and by bony warts in the lumpsucker (Cyclopteridae). Spiny scutes occur in the lateral line of the horse mackerel (*Trachurus*), along the midline of the belly of the herring (Clupeidae), and in other families. The threespined stickleback (*Gasterosteus*) carries a series of lateral plates in lieu of scales.

The vast majority of the teleosts are completely covered with scales; others are only partly covered (leather carp). The swordfish (*Xiphias*), when young, has tuberculoid scales but with growth they disappear and the larger individuals are scaleless. Most of the catfish families have no scales at all or have only soft dermal papillae such as are also found in some of the frogfishes (Antennariidae) and the clingfishes (Gobiesocidae). Some scales, as those of the eel, are microscopic in size; others may reach a length of two inches, as in the tarpon (*Tarpon*), or even more as in *Barbus tor* found in India. Some scales, usually cycloid, are *deciduous*, that is, they lie in shallow pockets and are easily rubbed off, as in the shiner (*Notropis*) and the smelt (*Osmerus*); others, especially

ctenoid scales, are more deeply embedded and difficult to remove, as in the yellow pikeperch (*Stizostedion*). In most teleosts the scales overlap. Those that are completely embedded in the skin, as in eels and also in the African and South American lungfishes (Dipnoi), may be more or less separated. In other species as in the burbot (*Lota*) even exposed scales may be isolated.

The type of bony covering has in general some influence on the activity of the fish. Individuals encased in armor cannot move with speed; they are usually sluggish; the armor serves as protection against pursuing enemies. Those with articulating scales have greater freedom of movement, and individuals with imbricating scales or a naked skin have even greater flexibility. Each of these changes from an immovable armor should make it possible for the individuals to increase their speed inasmuch as speed is largely dependent on the lateral movements of the body.

G. Modified Scales

Certain external organs found in fishes are essentially very similar to the scales in origin and structure. As a matter of fact, they are formed by a modification, enlargement, or fusion of the scales. Some show no resemblance whatsoever to a scale in appearance. Several examples are cited below.

In certain sharks as, for example, the spiny dogfish (*Squalus*) the single spines of the two dorsal fins are held to have their origin in the fusion of denticles. The same is true of the dorsal spine in the chimaeras. Such spines serve as defensive weapons especially when associated with poison glands. Another organ stated to have been formed from the enlargement or fusion of denticles is the grooved tail-spine or sting of the sting rays (Dasyatidae) and related species. It is generally serrated along both sides. The series of recurved hooks tear the flesh when the spine is withdrawn. The sting is definitely a protective structure. It is usually brought into action by an upward and forward thrust of the tail. In the surgeonfish (*Acanthurus*) scales at the base of the tail are modified into two knife-like spines that are carried in sheaths of skin but are ready to swing out in an attack.

The teeth found along both edges of the rostrum of the sawfishes (*Pristis*) are definitely modified placoid scales. The saw is used as an organ of defense and attack. It is especially effective when wielded sideways in a shoal of fish upon which the sawfish preys. Modified scales called stomodeal denticles line the buccal cavity and the pharyngeal box of the elasmobranchs (e.g. *Heterodontus*). Unlike the other organs mentioned above, they actually resemble a placoid scale in appearance.

They may serve to hold and grind food but are looked upon more as vestigial organs without a definite function. The teeth of many elasmobranchs are also specialized denticles. They have a pronounced resemblance to the typical placoid scales both in structure and appearance as, for example, in the spiny dogfish. When the papilla grows outside the buccal cavity, it forms a scale; when it passes over the ridge of the jaw inside, it develops into a tooth. In certain elasmobranchs the teeth are flat and plate-like, adapted for crushing or grinding, but their structure and that of the scales are still essentially identical.

Various other modifications occur. The squarish or oblong segments that form the jointed fin rays, the lepidotrichia, of the bony fishes are considered to be altered scales. They are virtually identical with the body scales in structure and they give support to the web of both paired and median fins. Again, in the shark, *Cetorhinus,* the long gill rakers of the branchial arches are said to be actually elongated, uncalcified denticles. Although they have the same function as the teleost rakers, their origin is entirely different.

Another type of modification is found in those scales that have been changed for a special function, although they still retain their identity as scales. An outstanding example is the lateral line scale that has been modified to provide an outlet to the surface for the mucus-filled, lateral line canal (see Vol. II, Chap. II). In such fishes as the yellow perch (*Perca*) the canal passes through an arch or tube located on the upper surface of the scale, the posterior opening of which provides an outlet. In the whitefishes (Coregonidae) the lateral line scale bears an elongated perforation, an open channel. In the pikes (Esocidae) the posterior field of this scale is deeply indented. In some forms the lateral line scale is only slightly altered providing an outlet through a simple pore. It is these modified, perforated scales that form the conspicuous line along the sides of most of the bony fishes.

In many teleosts an enlarged specialized scale, the axillary, is formed in the upper angle of the pectoral fin and in the outer of the pelvic. It usually has a pointed, dagger-like shape (e.g. *Elops*), and may either be stiff or flexible. In the Salmonidae, it is located in the adipose lobe of the pelvic fin. Its function is uncertain, although it may have some significance in locomotion.

There are other specialized scales among which may be mentioned the spine-like fulcra and the foricula. The fulcra line the anterior or dorsal rays of the paired and median fins in such fishes as the sturgeons and gars. They either form a double row along the edge or, as in the caudal fin of the sturgeon, the opposing scales may fuse into V-shaped fulcra. The function of the fulcra is not obvious but the foricula serves

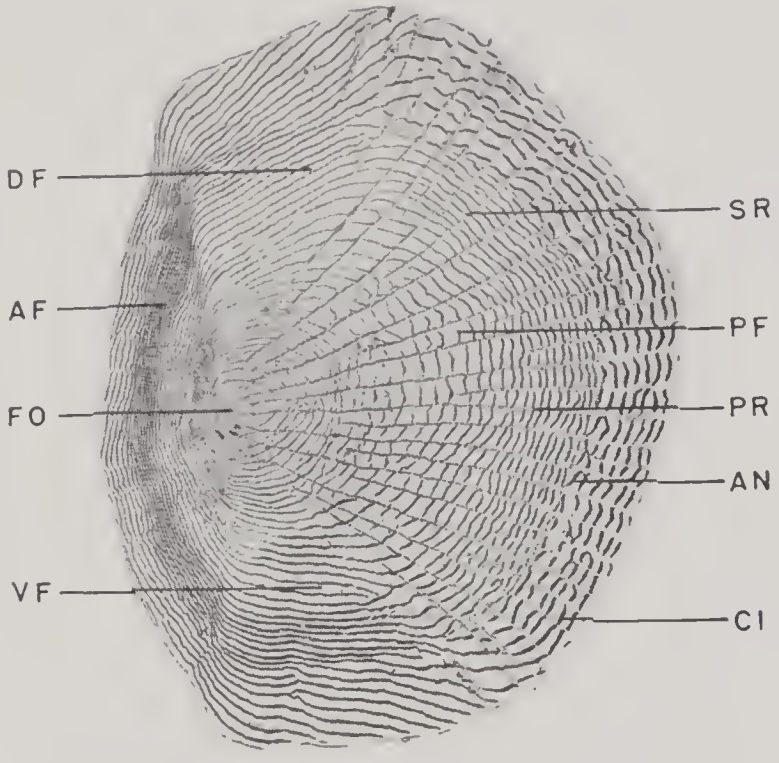
as a cover of the genital opening of the female four-eyed fish (*Anableps*).

Parenthetically, reference may be made here to the horny nuptial tubercles, warts, or granules called pearl organs that are commonly found on the head, fins, and scales of breeding males (sometimes in the females also) of many teleosts, especially the sucker (Catostomidae) and minnow (Cyprinidae) families. These temporary cornifications of the skin result from the hypertrophy of the cells of all layers of the epithelium. They are used chiefly as contact organs which enable the male to cling closer to the female during the spawning act when the bodies of the two sexes must come together in order to fertilize the extruding eggs. The roughness of the pearl organs counteracts the lubricating effect of the mucus, increases body friction, and thereby enables the fish to maintain better contact. The organs also serve some breeding individuals in combat and in nest building. After the spawning season the pearl organs peel off.

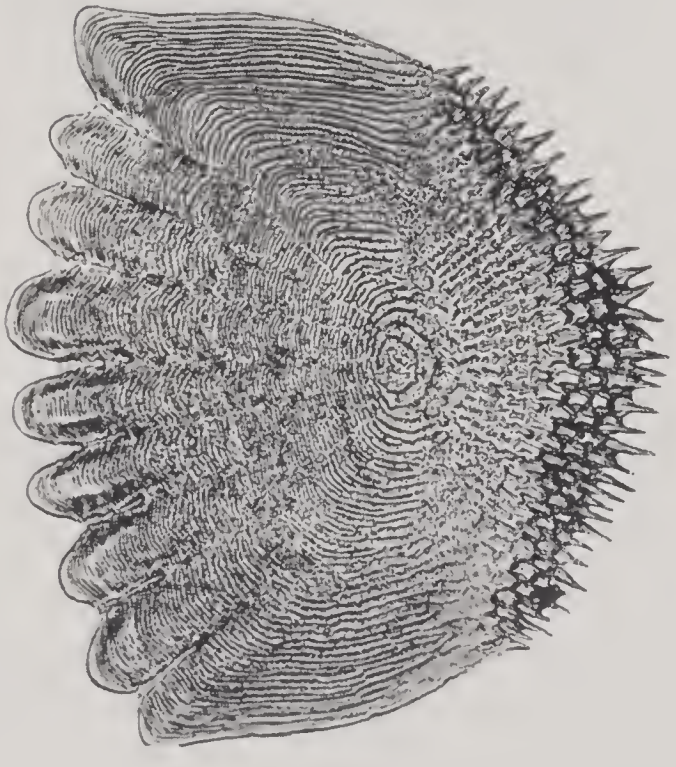
H. Use in Classification

Louis Agassiz classified fishes on the basis of their scales as Placoidei, Ganoidei, Cycloidei, and Ctenoidei. Although this system could not be adopted as such, the fact that it expressed in broad terms certain true phylogenetic and evolutionary relationships (classifications by earlier authors were more artificial) indicates that scales can be employed at least to some degree in the classification of fishes. Scales are of great systematic value in the study of fossil fishes. The fundamental scale characters are usually of little value in separating the smaller closely related taxonomic units. Scales can be of considerable importance in classifying the major groups, especially those within a restricted geographical area such as the Great Lakes of North America. In general, however, scales like many other structures have a limited use in systematics and their employment must be supplemented by a study of other anatomical characters. Only cycloid and ctenoid scales are utilized to any extent for diagnostic descriptions of living fishes. The surface features of the other types (placoid and ganoid) show little variability, except that the shape of the placoid scale and its spine may be characteristic of a species.

The scale characteristics utilized in classifications are the visible structures of the upper surface. Only fully formed scales are or should be employed. The histological features have little diagnostic values in living fishes except in distinguishing placoid, ganoid, and cycloid or ctenoid scales. In order to facilitate reference to a specific area, the outer surface of the scale as it is found in its normal position on the body is divided when possible into four roughly triangular *fields:* (Fig. 7A) anterior or basal, posterior or apical (in exposed region), dorsal,



A

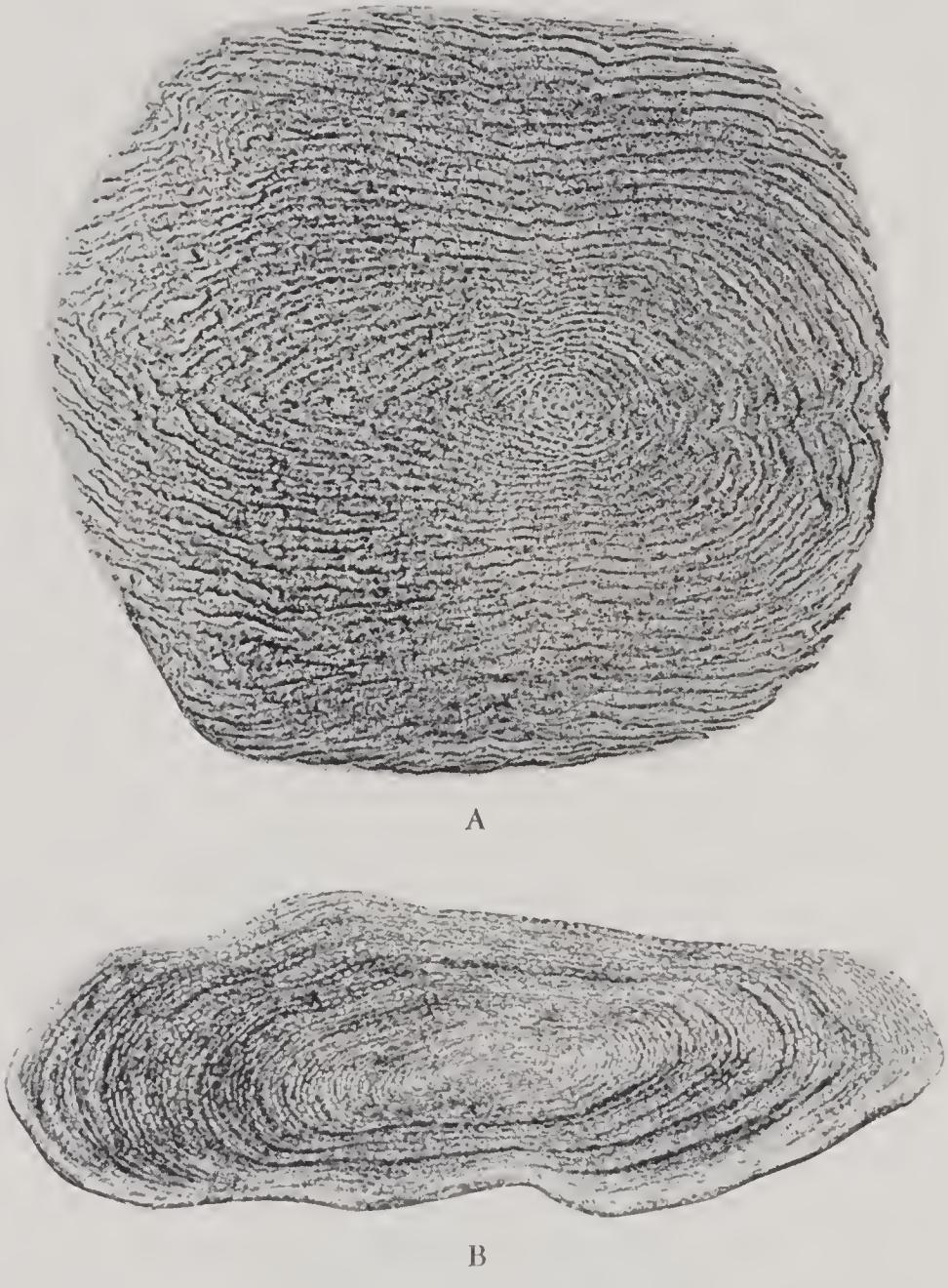


B

FIG. 7. A: cycloid scale (length 3.14 mm.) of the shiner, *Notropis cornutus*. B: ctenoid scale (length 3.50 mm.) of the yellow perch, *Perca flavescens*. DF, dorsal field; AF, anterior field; FO, focus; VF, ventral field; SR, secondary radius; PF, posterior field; PR, primary radius; AN, annulus; CI, circulus (ridge). (From K. F. Lagler.) Oriented with the anterior field to the left. The length of the scales was measured along the anterior–posterior diameter.

and ventral (lateral fields). These areas are often delimited by a more or less conspicuous angulation of the concentric ridges.

The main structures of the scale utilized in taxonomy are the *circuli*



A

B

FIG. 8. A: scale (length 3.85 mm.) of mudminnow, *Umbra limi*. B: scale (length 4.0 mm.) of American eel, *Anguilla bostoniensis* (from K. F. Lagler). Oriented with the anterior field to the left. The length of the scales was measured along the anterior–posterior diameter.

or *striae, radii* or *sulci,* and *ctenii.* The circuli are the relieved surface ridges that are continuous and homogeneous with the bony layer of the scale. They arise whenever the bone-forming materials occur in a quantity greater than can be utilized at the growing edge. They probably

function in anchoring the scale in its pocket. They are typically concentric, or nearly so, with the margin. In certain fishes as, for example, the clupeids the circuli are more or less transverse; in others such as the mudminnow (*Umbra*) (Fig. 8A) they are roughly longitudinal in relation to the antero-posterior axis. In the eel (*Anguilla*) (Fig. 8B) they are represented by a series of minute subquadrate bead-like elements termed loculi, formerly called platelets. The number of circuli usually

FIG. 9. Scale (length 10 mm.) of Atlantic salmon, *Salmo salar*, indicating four parr years in river (R.1–R.4), a full sea year (S.1), spawning (S.M.) in the second sea year, then a third full sea year (S.2), and part of a fourth (at the margin). The anterior field is directed upwards. (From S. T. Lindsay and H. Thompson, 1932).

varies with the fields, generally being the least numerous in the posterior area. The distribution pattern as well as the number is used in the classification of fishes.

Circuli mark successive stages in the growth of the scale. In many species the end of a year's growth is indicated by the approximation and discontinuity of the circuli. This area is termed an *annulus*. If retarded or a temporarily terminated growth is reflected in the scale at other times, that is, it does not represent an annual cycle, the area is designated an *accessory annulus*. A healed scar of an eroded margin caused by spawning activity is called a *spawning mark* (Fig. 9). Any other type of mechanical injury will also leave a scar after its repair. These several

features have in themselves no diagnostic value. However, many groups of fishes have a specific type or pattern of growth that is reflected in the spacings between the circuli and the distances between the annuli. Such a growth pattern may aid in identifying specimens from their scales.

Radii are grooves in the bony layer that originate at or near the center (*primary radii*) or some distance from it (*secondary radii*) and run toward the margin of the scale (Fig. 7A). In some species they tend to increase in number with age up to a certain point. Since the radii are open channels cut completely through the bony surface they give the scales a flexibility that enables them to conform to the shape and movements of the body. This function is aided by the increased pliability of that part of the fibrillary plate that lies directly beneath the groove. The principal diagnostic value of the radii lies in their presence or absence in the different fields. Radii may be entirely absent (Salmonidae) (Fig. 9) or incipient (*Labidesthes*). They may be present in only one field either anterior (*Esox*) or posterior (*Notropis*) (Fig. 7A), in both of these fields (Catostomidae) (Fig. 10), or in all four fields (*Barbus*).

Ctenii are tooth-like structures found in the posterior field of the ctenoid scale (Fig. 7B). They, like the circuli, form part of the surface layer of the scale, and may serve to hold the scale in place. Ctenii may appear in a single row parallel to the margin or in two or more rows; they may be prominent or weak; they may persist or wear down and disappear as the scale grows. These variations provide the diagnostic features of the ctenii.

The position of the *focus* or center of the scale with respect to its proximity to the various margins (usually the anterior and posterior), the shape (diamonded, circular, ovoid, squarish, oblong, etc.) of the scale, the presence or absence on the scales of prominent marginal lobes, scallops, or deep serrations (Fig. 7B), and the distribution pattern of the scales on the body may also have value in a systematic study. *Replacement (regenerated)* scales are not suitable for this purpose. Their central area of abnormal size that represents more or less completely the lost scale that was replaced is usually a blank, devoid of circuli and any other structures that are normally present. Lateral line scales are of considerable importance. Their number is noted in virtually all taxonomic studies as representative of the longitudinal series of scales. Other scale counts are made as follows: number of predorsal scales; number of rows on the cheek, above and below the lateral line, and around the body and caudal peduncle. The procedures employed in obtaining these values have varied with the investigators.

It should be emphasized that the various superficial structural details of scales differ markedly with the individuals of a species and even more

A

B

FIG. 10. A: scale (length 2.78 mm.) of sturgeon sucker, *Catostomus catostomus*. B: scale (length 1.38 mm.) of burbot, *Lota lota* (from K. F. Lagler). Oriented with the anterior field to the left. The length of the scales was measured along the anterior–posterior diameter.

so with the several areas on the body of the same fish. The size and form seem to depend a good deal on body location. For example, a noticeable difference is often seen in the shape of the scale from the cheek, fin bases, back, breast, belly, sides, and caudal peduncle. In many species the scales are cycloid in the young and ctenoid in the adult; cycloid on the cheek and fin bases and ctenoid on the trunk. Considerable variability also occurs with growth and age in the shape of the scale, the number of radii, circuli, and ctenii, in the spacings between the circuli, and, of course, the distance between and the number of annuli. Nevertheless, in spite of all this variability there does exist a fairly distinct, diagnostic type of scale in various groups of fishes (see Figs. 7–10). To identify and describe the diagnostic features requires an examination of many scales from different parts of the body and from many individuals. Only typical scales can be used with confidence in systematic studies.

I. Use in Life History Studies

Cycloid and ctenoid scales have also proven to be highly serviceable in estimating the age and growth of fishes (see Chap. IX). Those species that inhabit the temperate waters and undergo an annual cycle of growth are most apt to reflect this growth on their scales. In tropical waters where seasonal differences in growth are not marked the zones of growth on the scales if present are poorly defined.

As already indicated (p. 237) seasonal and annual increase in body length may be reflected on the scales. Once the full complement of scales has been formed their number remains practically fixed except for the occasional intercalated scales (p. 228). Since the growing body remains completely covered, the scales must of necessity increase in size. During this period of growth the scales add their circuli or growth rings to the margin. In many species the distance between the circuli varies with the intensity of growth. In such instances when growth slows down, the circuli become weak, close set, and discontinuous. In some species arrested growth is indicated only by a discontinuity or break in the ridges. Cessation of body growth ultimately ends in suspension of scale formation.

In the temperate zone pronounced retardation of growth takes place normally once a year, usually in the fall, followed by a period of complete rest. When growth is resumed, the new more or less complete circulus or wide-spaced circulus forms the margin of the area of retarded growth (annulus). Thus each year a growth zone, bordered by discontinuous or approximated circuli, develops on the scales, the number of these zones representing the age of the fish in years.

Any marked retardation in or cessation of body growth irrespective of

the cause will be registered on the scales when normal growth is resumed. If such an occurrence takes place at a time other than that of annulus-formation an extra or accessory check will be left on the scales. Such a mark may result from a disease, parasitization, an injury, starvation, sexual maturation, pollution, drop in temperature, or any other prolonged unfavorable change in the external and internal conditions of life.

An accessory mark need not necessarily be found on all scales of an individual. When a group of lost scales is being replaced the growth of the scales adjacent to the denuded area is usually retarded until the fast-growing replacement scales assume a normal growth. Scales remote from the denuded area are not affected. An accessory mark is not always recognizable as such so that erroneous estimates of age and growth may be obtained. An identifiable accessory check common to most of the scales testifies to the occurrence of some untoward event in the life of the individual; if found on only a few scales a local body disturbance is indicated.

In maintaining coverage of the body the scales have been found to grow at an approximately fixed ratio with the fish. The growth of the scale, therefore, is more or less a replica of that of the body. This fact has made possible the employment of scales in estimating the past growth of an individual. Multiplying the ratio of the length of that part of the scale that was completed at the end of a certain year of life to the final length of the scale by the length of the fish gives the estimated length that the individual attained at that particular age. Yearly increments of growth can be obtained by subtracting the different computed lengths.

Body and scale seldom grow in an absolutely fixed ratio but it is almost always constant enough from one year's end to another to provide fairly accurate computations of growth. However, a corrective factor can be applied to the calculations. If samples of fish are not fully representative of an entire year class then factors other than or in addition to disproportionate growth between body and scale enter into any discrepancies in the average calculations of growth. Samples seldom are wholly adequate and consequently computations of the average growth of a year class are actually approximations.

Some fishes show no definite annuli on their scales; others have no scales at all. The age and growth of these species may usually be determined from other structures such as the otolith, operculum, fin-spine, vertebra, or other bony parts. These structures sometimes reveal the year marks much more clearly than the readable scales.

An excellent example of the revelation of the life history of a species

from its scales is found in the Atlantic salmon (*Salmo*). Figure 9 shows a scale from a 10-pound female salmon. This fish spent four years (R. 1–R. 4) as a parr in the river, then it went to sea and in the second year there returned to spawn (S.M.), and after one more complete year (S. 2) and part of another in the sea it again entered the river where it was caught before the second spawning at the age of 8 years.

Age and growth are extremely valuable indices of the environmental conditions of fishes, and their determination plays an important part in the management of the fisheries and in fish cultural practices. An analysis of age composition is basic to a study on the relation between the strength of year classes and the abundance of fish. This knowledge facilitates further investigations on the factors of abundance, and makes possible a forecast of the prospects of fishing in future years. Age determinations permit studies on variation in growth rates with species, latitudes, and different bodies of water, and again facilitate investigations of the factors involved. By observing the time of formation of the annuli, the length of growing seasons may be determined. Age and growth studies reveal whether poor fishing in a stream or lake is the result of slow or stunted growth or a short life span of the fish or of a paucity in numbers that demands a stocking program. The success of a planting program can be checked when it is known at what age most of the individuals first enter the catch.

A knowledge of age and growth is also important in systematics, taxonomy, and racial investigations, especially where results depend on the dimensional relationships of different parts of the body. The number of lateral line scales, usually employed as a diagnostic character, varies with the length of the individual within a species at the time of completion of squamation, and if a collection is dominated by individuals that were slow growing as juveniles the number of scales recorded will be below the true average. Again, the relative lengths of the head, fins, jaw, and eyes, also employed in systematics, have been observed to decrease with increase in rate of growth. Comparing fish of the same length, as is often done, is no safe check if they had widely divergent growth rates or were of different ages. As already indicated (p. 238), growth rate itself may differentiate races and even species, such as the deep-water ciscoes (*Leucichthys*) of the Great Lakes that are difficult to identify by the usual taxonomic characters.

In investigations of predator–food relationships it is of common occurrence to find that the fish consumed has been digested to a point of unrecognizability or doubtful identity. Scales are usually the last of the ingested materials to disappear from the stomach and intestines and

often leave the only clue to the identity of the species used as food. A similar situation exists for the fish predators that leave a midden heap or dunghill containing scales.

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CHAPTER VI

ENDOCRINE ORGANS[1]

WILLIAM S. HOAR

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[1] Dr. Grace E. Pickford read this chapter in manuscript and made numerous additions and suggestions which I have used freely in making the revision.

I. INTRODUCTION

Fishes possess a well-developed endocrine system comparable with that of other vertebrate animals. The recent demonstration of tissue with parathyroid-like function (Rasquin and Rosenbloom, 1954) has filled a gap formerly thought to exist in the list of fish endocrine organs. The dominant position held by the pituitary gland, through its different tropic hormones, has been established by careful studies of the effects of hypophysectomy and other endocrine relationships in *Fundulus* (Pickford *et al.*, 1953). In the endocrine imbalance and tissue hyperplasia

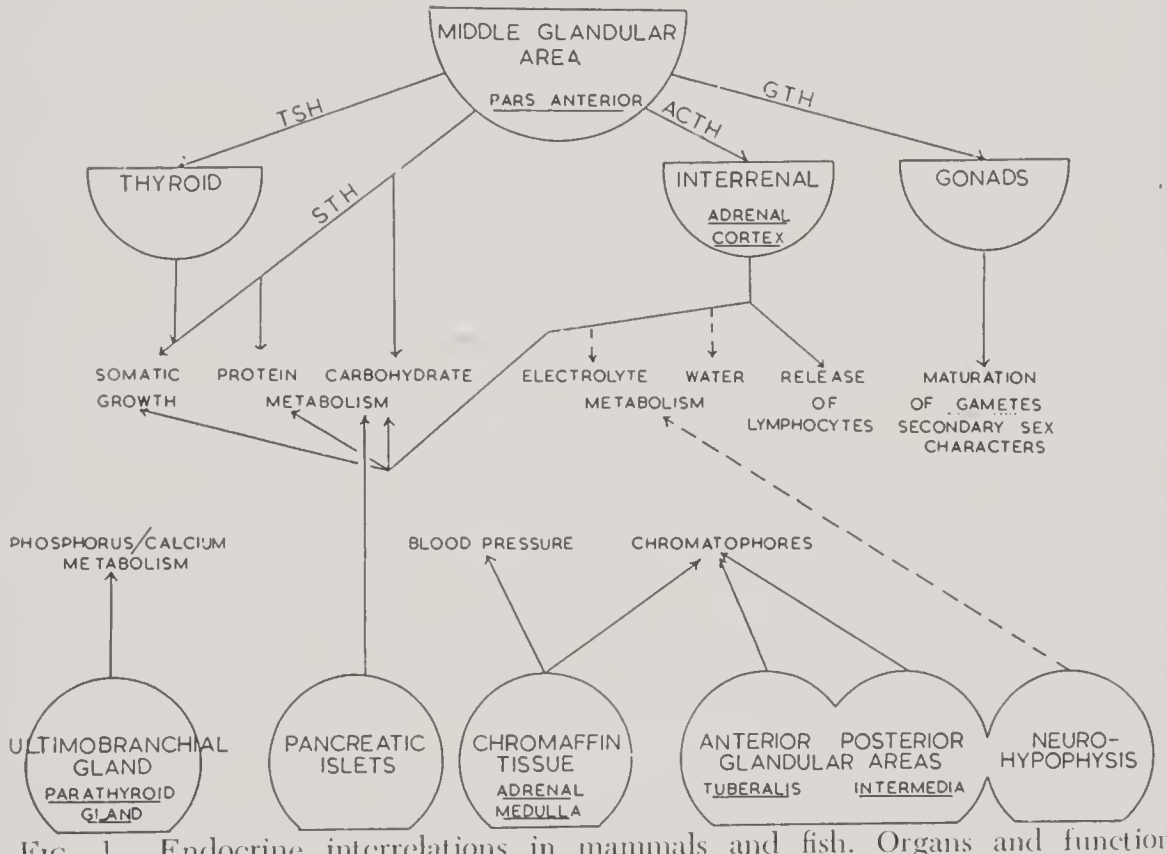

FIG. 1. Endocrine interrelations in mammals and fish. Organs and functions dominated by the pituitary are shown at the top. Broken lines indicate relationships recognized in some vertebrates but not demonstrated in fish. Modified after Rasquin and Rosenbloom (1954).

which develops when *Astyanax* is maintained under adverse conditions. Rasquin and Rosenbloom (1954) have found convincing evidence that chemical regulation in fishes and the interrelationships of the endocrines with target organs follow the general pattern recognized in mammals. Major contributions to the endocrinology of fish have also been made in studies of the physiology of migration (Fontaine, 1954; Olivereau, 1954) and reproduction (Bretschneider and Duyvené de Wit, 1947; Dodd, 1955; Hoar, 1951, 1955). For purposes of orientation a comparative summary showing the interrelations of endocrines in mammals and fishes is presented in Fig. 1.

II. ANATOMICAL RELATIONS AND GENERALIZED STATEMENT OF FUNCTION

A. Pituitary

The arrangement and organization of the component parts of the pituitary gland varies greatly in different species (Fig. 2). Olivereau

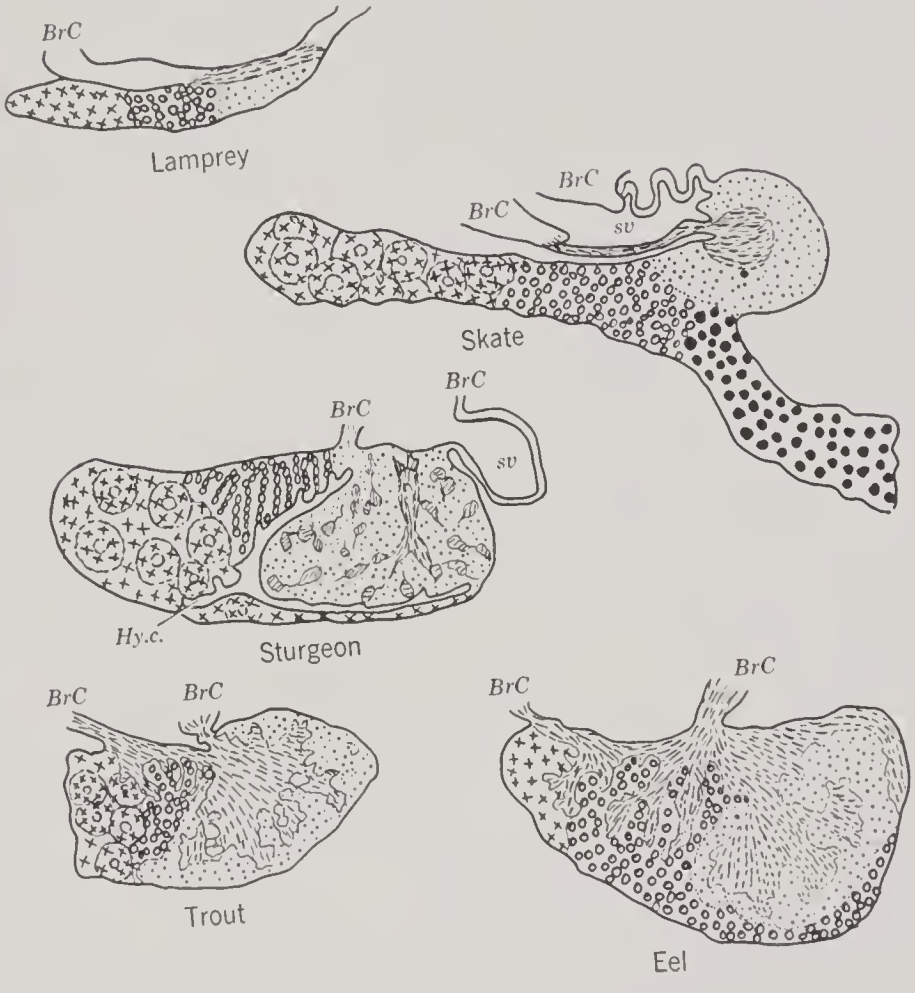

FIG. 2. Diagrammatic mid-sagittal sections of the pituitary gland. Crosses, anterior glandular region; open circles, middle glandular region; stippling, posterior glandular region; short irregular lines, pars nervosa; solid circles, ventral lobe of elasmobranch gland; BrC, brain connection; SV, saccus vasculosus; HyC, hypophysial canal. Similarly depicted areas may not be physiologically comparable in cyclostomes, elasmobranchs, and teleosts. Lamprey redrawn from de Beer (1923); skate from Howes (1936) with permission of the Quarterly Journal of Microscopical Science; and sturgeon, trout, and eel from Kerr (1942, 1949) with permission of the Zoological Society of London.

(1954) has recently discussed the terminology and the homologies of the different areas. Distinct neurohypophysis and adenohypophysis are always present. The neurohypophysis or pars nervosa is closely asso-

ciated with the posterior portion (pars intermedia) of the adenohypophysis, and the term neuro-intermediate lobe is often applied to this region. In teleosts strands of tissue from the neurohypophysis may also extend into the other regions of the adenohypophysis.

In teleosts the adenohypophysis is composed of three parts: posterior, middle, and anterior glandular regions. Of these, the posterior glandular lobe is homologous with the intermediate lobe of the mammalian gland. The middle glandular region (transitional lobe; *Ubergangsteil*) contains cell types and produces hormones characteristic of the anterior lobe of higher vertebrates. The homologies of the anterior glandular portion, however, are not clear. Some writers have compared it with pars tuberalis while others have considered it a part without equivalence in the mammals. Olivereau (1954) reserves judgment and, until more is known of its physiology, this would seem to be wise.

Hypophysectomy and injections of hormone extracts have demonstrated the existence of fish pituitary growth hormone (somatotropin), gonad stimulating hormone(s) (gonadotropin), thyroid stimulating hormone (thyrotropin), and adrenal cortical stimulating hormone (adrenocorticotropin ACTH). There is reason to believe that these activities are associated with the middle lobe. This is in line with the supposed homologies of this region. Diabetogenic activity (believed to be associated with growth hormone in mammals) has also been demonstrated in fish pituitary extracts (Houssay and Biasotti, 1931). It is possible that the fish pituitary may contain an erythropoietic factor since hypophysectomized *Fundulus* are anemic and this anemia is not corrected by purified growth hormone (Pickford, 1953a, 1954a).

The chromatophore-dispersing hormone, intermedin, has long been recognized in fish pituitary and is certainly associated with the posterior lobe. A melanophore concentrating hormone is present in the anterior regions of the gland (Healey, 1940, 1948), possibly in the anterior lobe itself. Confirmation of this hypothesis would support the view that this region is homologous with the tuberalis, since it is suspected that the melanophore-concentrating agent of amphibians is associated with this region (Hogben and Slome, 1931). The physiology of the chromatophorotropins is discussed in Vol. II, Chap. VIII.

It is extremely doubtful whether the hormones of the pars nervosa, oxytocin and vasopressin, are present in elasmobranchs (Waring and Landgrebe, 1950), although minimal amounts have been claimed by some investigators (Heller, 1950). On the other hand the teleostean hypophysis is rich in these hormones. Their role in the group, however, is still problematic (Heller, 1950). Wilhelmi *et al* (1955) have found that the S-shaped spawning reflex of *Fundulus* is elicited by posterior

lobe hormones, whereas gonadotropins and other anterior lobe preparations have little or no such activity.

Some headway has been made in localizing cells and areas responsible for the different pituitary hormones. Correlations of growth rate or sexual development with abundance of cell types suggests that acidophils produce somatotropin while basophils produce gonadotropin (Olivercau, 1954). Periodic acid–Schiff staining has demonstrated two types of basophils in the middle glandular area of the pituitary of *Astyanax mexicanus*. One is believed to be thyrotropic and the other gonadotropic (Atz, 1953). Adrenocorticotropin production has also been attributed to basophils of this area in *Astyanax* (Rasquin and Rosenbloom, 1954).

Available information on comparative biochemistry of the pituitary hormones suggests marked differences in chemical constitution and/or relative potency among different vertebrates. For example, fish respond well to mammalian somatotropin and thyrotropin (Leloup, 1952; Pickford, 1954a, b) but mammals, when treated with fish preparations, show little response in growth (Pickford, 1954a) or thyroid activity (Gorbman, 1946; Fontaine, Y.-A., 1954). On the contrary, fish are relatively unresponsive to mammalian gonadotropins (Hoar, 1955). Mammalian ACTH readily activates the interrenal of fish (Dittus, 1940; Rasquin, 1951) and extracts of elasmobranch interrenal prolong the life of adrenalectomized rats (Grollman *et al.*, 1934). At the present stage it can only be concluded that marked differences occur among classes and species in response to comparably prepared extracts and much additional work will be required before generalizations are possible. The hormones of the fish pituitary may be specialized in association with the problems of aquatic life or may show a stage in the biochemical evolution of the more elaborate array of endocrines recognized in mammals. This point is discussed again in connection with the hormones of reproduction.

The mutual interaction between the pituitary gland and target organs is apparent in many of these studies. The matter was carefully considered by Dittus (1940) in his comprehensive work on the interrenal and again by Egami (1954a, b), who showed that decreased output of gonadotropins followed administration of estrogen or androgen, and by Rasquin and Rosenbloom (1954) in their studies of *Astyanax*.

B. Thyroid

The endostyle of the ammocoetes constitutes the most primitive thyroid mechanism for the conversion of accumulated iodine to thyroid hormone (Leloup and Berg, 1954). At metamorphosis this structure forms typical

thyroid follicles showing clearly the phylogenesis of the vertebrate gland (Goldsmith, 1949). In elasmobranchs and a few teleosts the follicles are organized in a compact encapsulated organ but in other fishes they are widely scattered about the ventral aorta and must be studied from histological sections of the throat region (Fig. 3). Thus, it is impossible to perform surgical thyroidectomies in most fish and the physiologist must rely on histophysiological studies, chemical thyroidectomies, and

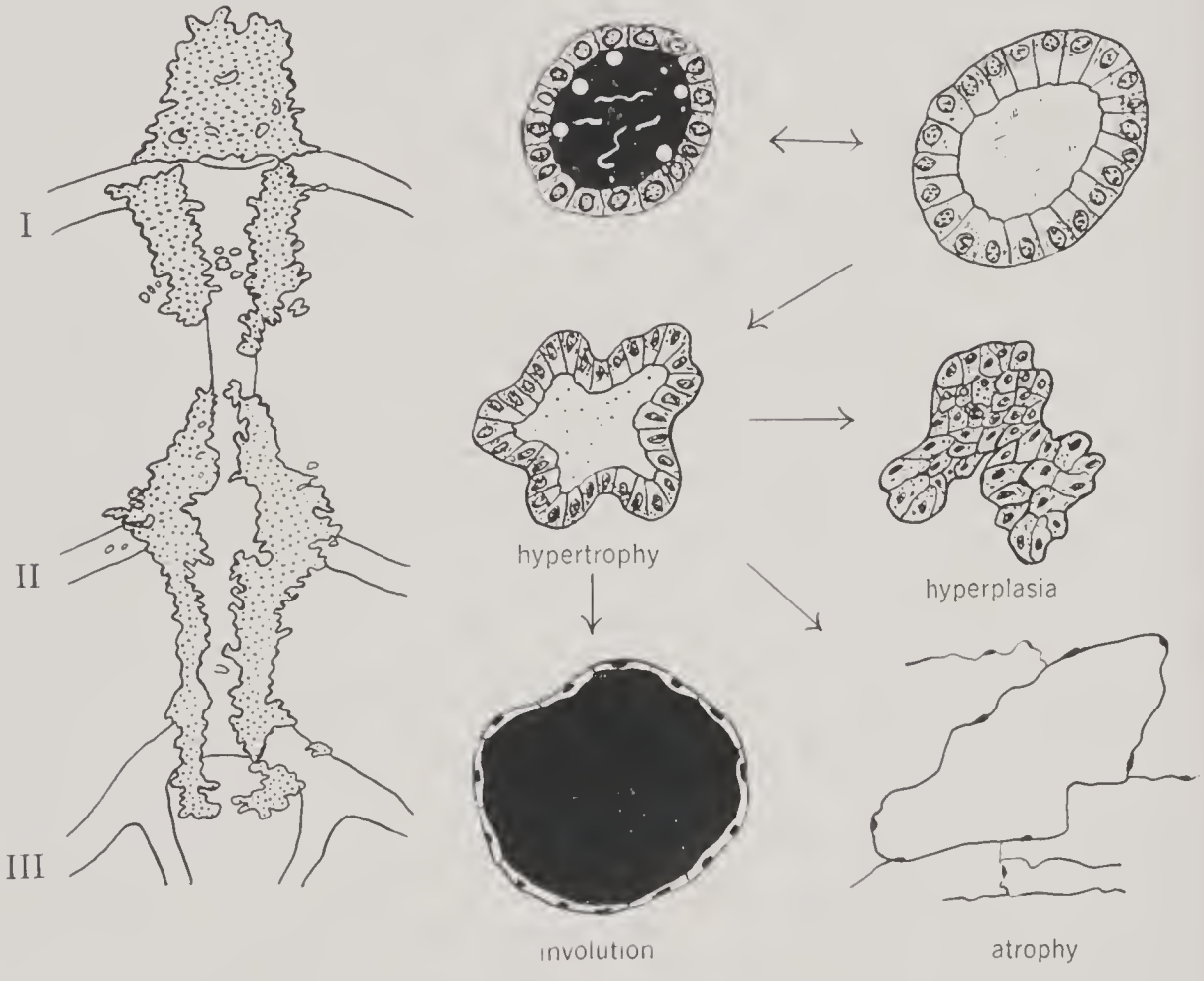

FIG. 3. The teleost thyroid. Left, graphic reconstruction of thyroid tissue (stippled) of Atlantic salmon in relation to the ventral aorta and its branches (I to III), dorsal view; right, appearance of typical thyroid gland follicles in different stages of activity. Drawn from photomicrographs by Hoar (1939, 1952), Olivereau (1954), and Rasquin and Rosenbloom (1954).

tracer techniques. With radio-iodine it has been possible to achieve a total destruction of the thyroid gland (La Roche and Leblond, 1954).

The available evidence suggests that thyroid cells capture inorganic iodine from the blood, store it in the form of thyroid hormone in the follicles, and enzymatically release it later into the blood. Monoiodotyrosine, diiodotyrosine, and thyroxine have been identified in fish and present evidence indicates that thyroid hormone is identical or at

least similar throughout the vertebrates. Production of triiodothyronine by fish has not been definitely established (Gorbman, 1955).

There seems to be a fundamental difference between poikilotherms and homoiotherms in the reaction of their target organs or processes to thyroid hormone. Morphogenetic effects and effects on activity are read-

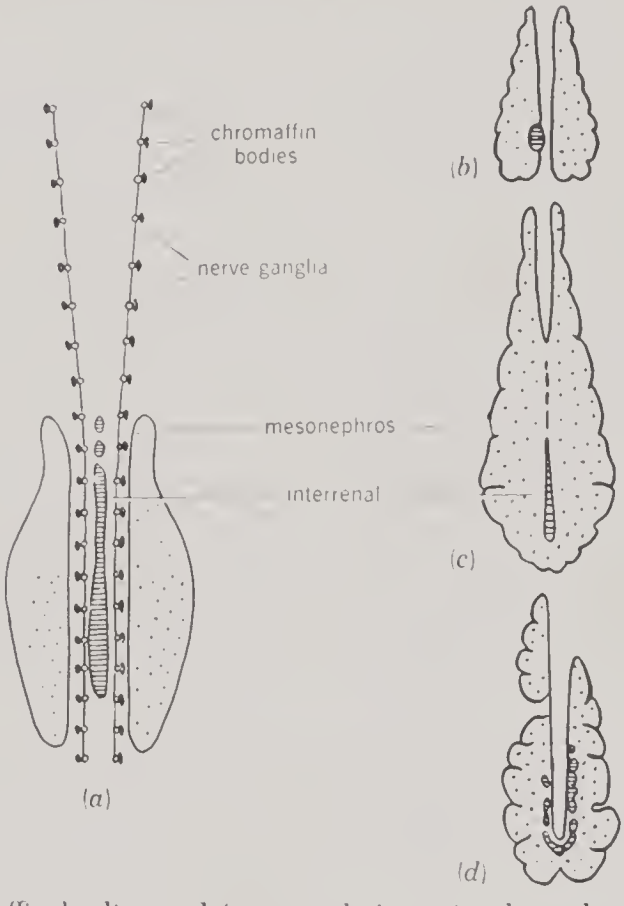

FIG. 4. Chromaffin bodies and interrenal tissue in elasmobranch fishes. a: general distribution after Vincent (1922); b: *Scyllium* (*Scyliorhinus*) type; c: *Raja* type; d: *Torpedo* type. Dorsal views. From Dittus (1940) with permission of the Editor of *Z. wiss. Zool.* In *Torpedo* the interrenal mas is occasionally located on the right kidney and sometimes shared by the two kidneys.

ily demonstrated (Dales and Hoar, 1954; Hoar *et al.*, 1955) so that a calorigenic effect of thyroxine would be expected in fish as in higher vertebrates. The two most recent investigations, however, have produced contradictory results. Müller (1953) in a careful investigation showed increased oxygen consumption of goldfish treated with thyroxine or with anterior lobe preparations rich in thyrotropin. Matty (1954), on the other hand, showed that surgical thyroidectomy has no effect on the respiration of elasmobranchs. This much investigated and still puzzling problem is discussed in the section on metabolism. The way in which thyroid hormone enters the biochemical chain in the metabolism of different vertebrates remains one of the most perplexing problems of comparative physiology.

C. Chromaffin Tissue

Scattered chromaffin tissue (Figs. 4 and Plate I, Fig. 3) of neural origin is intimately associated with the sympathetic ganglia both in location and function. Tests with extracts of chromaffin tissue of *Lampetra fluviatilis* (Gaskell, 1912) and several elasmobranchs (Lutz and Wyman, 1927) suggest that the secretions of these cells are similar physiologically to those of the mammalian adrenal medulla, exercising a general sympathomimetic effect. The chromaffin bodies of elasmobranchs contain a high percentage of noradrenaline (Shepherd *et al.*, 1953). As in the case of thyroid hormone, however, the responses of the target organs to adrenaline seems to differ among vertebrates. This is perhaps to be expected since the autonomic nervous system shows fundamental variations with progressive increase in specialization through the three major classes of fish-like vertebrates (Nicol, 1952). The action of adrenaline on different tissues and processes is discussed in other chapters.

Variations in the distribution of this tissue among fishes are of phylogenetic interest. In the majority of elasmobranchs, alone among vertebrates, the chromaffin (adrenaline-producing) tissue is completely separated from the interrenal (cortical steroid-producing) tissue (Vincent, 1922). The two components are more or less closely associated in teleosts and, although some species such as *Pleuronectes*, retain a primitive arrangement (Krauter, 1951), the two tissues usually intermingle. This process reaches a climax in *Cottus* where chromaffin cells are localized within the interrenal tissue and form an adrenal complex similar to that of amphibians (Baecker, 1928).

D. Interrenal Tissue

Baecker (1928) and Dittus (1940) have described the interrenal tissue of many fishes. Aboim (1946) has made an extensive review of the literature. The elasmobranch interrenal is located posteriorly in one or more compact masses between the opisthonephroi and is discreet enough to permit surgical removal (Fig. 4). In teleosts the interrenal must be located histologically. Here it is confined to the cranial regions of the head kidney and usually forms a sheath of one or more layers of acidophilic cells around the posterior cardinal veins and their branches (Plate I, Fig. 1). In the lungfish, *Protopterus*, interrenal and chromaffin cells intermingle and are located along the large venous capillaries ventral to the kidney (Gérard, 1951).

Only one cell type has been described. Dittus (1940) compared the different functional stages in the interrenal cells of elasmobranchs with the zones in the mammalian cortex. Rasquin (1951) found that histochemical methods used for ascorbic acid and lipid inclusions in the

mammalian cortex gave unsatisfactory results on fish tissue. Using chemical methods, Fontaine and Hatey (1954b) found abundance of ascorbic acid in the interrenal region of both selachians and teleosts.

The interrenal tissue seems to produce indispensable cortical steroids. Interrenalectomy has often been performed in elasmobranchs and, as in

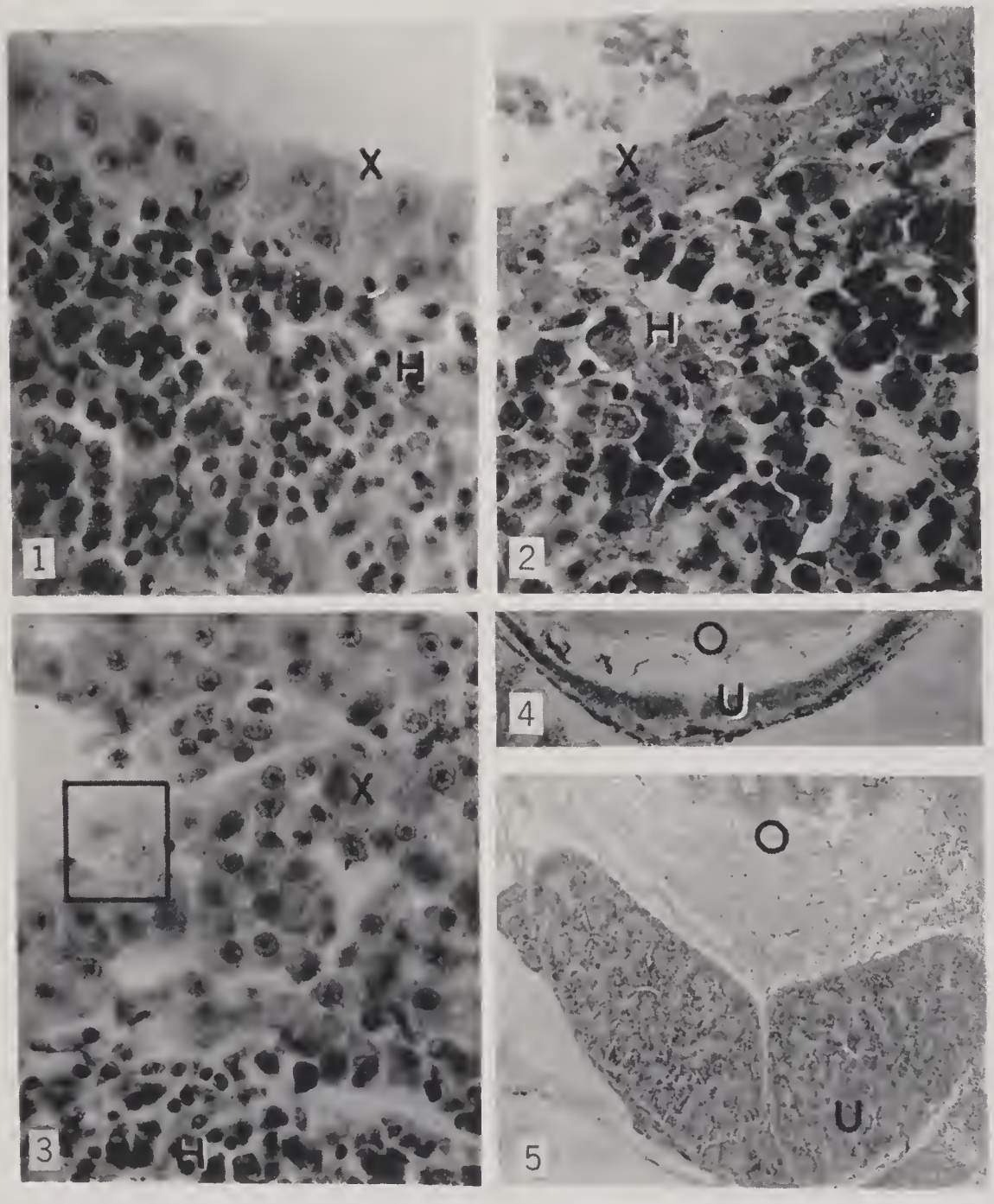

PLATE I. Figs. 1–5. Photomicrographs of interrenal and ultimobranchial tissue of *Astyanax mexicanus* by Rasquin. Fig. 1, normal interrenal. × 1020. Fig. 2, atrophied interrenal. × 1020. Fig. 3, interrenal hypertrophied with ACTH; two chromaffin cells are circled. × 850. Fig. 4, ultimobranchial gland, normal. × 115. Fig. 5, ultimobranchial gland, hypertrophied. × 115. X, interrenal tissue; H, lymphoid tissue of head kidney; O, esophagus; U, ultimobranchial gland.

mammals, is invariably followed by death (Hartman *et al.*, 1944). Moreover, it has been shown that extracts of the selachian interrenal maintain life and growth of adrenalectomized rats (Grollman *et al.*, 1934). Finally the histological response of the interrenal of fish under "stress" and its hypertrophy following injections of ACTH suggest comparable adrenal cortical mechanisms in fishes and tetrapods (Dittus, 1940; Rasquin and Rosenbloom, 1954).

Three biological groups of adrenocortical steroids are recognized in mammals: (1) the sex hormones, especially androgens; (2) the 11-deoxycorticosteroids regulating electrolyte metabolism; and (3) the 11-oxycorticosteroids related to carbohydrate metabolism (Bishop, 1954). Present evidence suggests that the cortical steroids of the elasmobranch interrenal function in carbohydrate but not in electrolyte metabolism (Hartman *et al.*, 1944). In this group, histological evidence (Dittus, 1940) of changes in interrenal activity during the sexual cycle indicates an interrelationship between gonadal and cortical steroids. The situation in teleosts is probably similar to that in elasmobranchs (Atz, 1953). Chemical analyses have shown 17-hydroxycorticosteroids and 11-oxycorticosteroids in the blood of salmon (Fontaine and Hatey, 1954a, b, c; Hatey, 1954) but no one seems to have reported on the deoxycorticosteroids in fish. However, the survival of adrenalectomized mammals treated with extracts of elasmobranch interrenal tissue (Grollman *et al.*, 1934) suggests that fish produce a variety of adrenocortical steroids whether or not the "mineralcorticoid" effect is physiological in this group of vertebrates.

In fish, as in mammals, "stress" (Selye, 1949) produces a characteristic response on the part of the pituitary and the interrenal (adrenal cortical) organ. The degenerative changes produced in *Astyanax* (Plate I, Fig. 2) maintained in constant darkness are strikingly similar to those recognized in mammals under "stress" (Rasquin and Rosenbloom, 1954). Other studies support this general conclusion. Thus the work of Rasquin (1951) on *Astyanax* showed a prompt involution of the lymphoid organs following administration of ACTH. In the perch, Bisset (1949) has shown that administration of mammalian adrenal cortical extract stimulates the release of antibodies in previously immunized animals.

E. Gonads

Gonadectomized fish fail to develop the secondary sex characters and behavior displayed by intact animals. This operation has now been performed on many species and the results show that the development and expression of many of the sexual characters is dependent upon gonadal hormones. In addition to surgical castration, which is frequently

followed by regeneration, the total destruction of the gonads may be effected with x-rays (Follenius, 1953) or by treatment with radioactive phosphorus (Vivien, 1953). Recent reviews by Dodd (1955) and Hoar (1955) contain references to the pertinent literature.

Some workers have argued that interstitial cells of Leydig, because of their development and presence at the time of sexual maturity, are responsible for the male hormones. Other workers have failed to find interstitial tissue in sexually mature fish or have not been able to correlate its development with sexual maturity. Marshall and Lofts (1956) find two distinctly different arrangements of endocrine tissue in fish testes. The typical vertebrate interstitial gland (Leydig cell) pattern is evident in such fish as *Gasterosteus aculeatus*, *Tilapia* spp., *Clupea sprattus*, *Latimeria*, *Scyliorhinus* and *Chimaera*. On the other hand, fish such as *Esox lucius*, *Salvelinus willughbii* and *Labeo* (sp. ?) lack the secretory interstitium but possess "lobule boundary-cells" which are histochemically similar to the Leydig tissue. Between breeding seasons the walls of the seminiferous tubules are apparently bounded by fibroblasts. Prior to the breeding season these are modified into lipoidal cholesterol-positive gland cells. Like the typical Leydig cells these lobule boundary cells expend their cholesterol-positive material prior to spawning. Interstitial and lobule boundary cells are morphologically similar and may be presumed to perform the same function. Their phylogenetic relations have not yet been established.

Mclampy and Cavazos (1954) have made a comparative histochemical study of lipids in the testis of a series of vertebrates, including the teleost *Lepomis*. In this species lipids were found only in the germ cells and interlobular sheath. Ketosteroids could not be demonstrated in the Leydig cells and Sertoli cells were said to be absent. Sertoli cells are, however, found both in elasmobranchs (Fratini, 1953) and teleosts (Craig-Bennett, 1931; Follenius, 1953). They may secrete hormones as in higher vertebrates (Teilum, 1950). At present it is impossible to localize definitely the site of testis hormone formation.

The physiology of the ovarian follicular epithelium and hormone production by the ovary are discussed in Chap. VII. Postovulatory corpora lutea reach a high degree of differentiation in viviparous elasmobranchs. It seems probable that they produce hormones but the chemical nature of the secretions has not been established.

F. Islets of Langerhans

Barrington (1942a) has shown that, in the ammocoete larva, several small masses of cells (follicles of Langerhans) embedded in the wall

of the intestine at the junction of the fore-and mid-gut are physiologically comparable to the islet tissue of other vertebrates. Similar tissue has been identified in adult cyclostomes (Barrington, 1945). In these primitive fishes only one type of cell is distinguished and this bears no clear resemblance to any of the three cell types usually found in islet tissues. Despite atypical staining reactions, however, there can be no doubt that these cells correspond to the beta (insulin-producing) cells of higher vertebrates. Injections of glucose produce characteristic vacuolization of the cytoplasm, and destruction of the follicles by cautery leads to a significant rise in blood sugar.

In elasmobranchs the islet tissue is embedded in a compact pancreas. The primitive condition, seen in the majority of species, consists of a second epithelial layer surrounding the smaller ducts. In some species cords of cells extend into the surrounding exocrine tissue and in *Squalus* many of these cords become separated off as islets (Thomas, 1940).

In teleosts small masses of islet tissue are located in the region of the gall bladder, spleen, pyloric caeca, and small intestine. These may or may not be anatomically related to the zymogenous tissue of the diffuse pancreas. There is usually one principal islet mass and in some fish the islet tissue is concentrated in a single compact organ either capping the gall bladder or located near to it (Rennie, 1904; McCormick, 1924). In both elasmobranchs and teleosts two major cell types are recognized, similar to the alpha and beta cells of mammals. In some species other categories have been described (Clausen, 1953). Cobalt chloride, which destroys the alpha cells, and alloxan, which destroys the beta cells in mammalian islets, have been used in fishes with results comparable to those described in other vertebrates. In fish, as in mammals, the insulin-producing tissue is rich in zinc (Weitzel *et al.*, 1953). These findings indicate similar or identical hormones in the islets of different vertebrates. Details are given in the section on carbohydrate metabolism.

G. Ultimobranchial Gland

A parathyroid-like function has recently been attributed to the ultimobranchial gland. This small epitheloid mass of cells (Plate I, Fig. 4) develops from the pharyngeal epithelium at the level of the last branchial fissure and, in adult fish, is located between the ventral wall of the esophagus and the sinus venosus. Krawarik (1936) described the somewhat variable histological architecture in 31 species of fish, mostly teleosts. The gland does not seem to have been located in cyclostomes (Watzka, 1933).

Rasquin and Rosenbloom (1954) have summarized the literature and

described the normal and hypertrophied ultimobranchial gland in *Astyanax mexicanus* (Plate I, Figs. 4, 5). This interesting investigation is based on a careful study of the histology of the endocrine organs of the eyed forms of *Astyanax mexicanus* maintained in total darkness. Continuous darkness is detrimental and produces a "stress" which is evident in the endocrine imbalance observed. An examination of the ultimobranchial glands of almost 100 fish raised in darkness for periods of from 4 months to 2½ years revealed a high correlation between hypertrophy of this tissue, pathological changes in the kidney, and a fibrosis of the skeletal elements. Distortion of the vertebral column, caused by loss of calcium and subsequent fibrosis of bone, was observed, while degenerating tubules, calcium deposits, granular deposits, and fibrosis were found in the kidney. The authors seem justified in postulating a parathyroid-like function for the ultimobranchial gland.

The nature of the hormone is unknown. At least one attempt has been made to study the effects of mammalian parathormone on fishes. A. J. Rampone, working in this laboratory, injected goldfish with from 1 to 30 units of parathormone (Lilly) and found no significant change in the bone or muscle ash. Eighty-two goldfish were injected and analyzed with comparable number of controls and temperatures were varied from 15 to 30° C. in different experiments. The methods used were adequate to show changes in mice injected with 2 units.

H. Other Hormones and Tissues with Possible Endocrine Function

1. Secretin and Gastrin

Several investigators have found that humoral rather than nervous mechanisms are responsible for the regulation of digestive functions (Barrington, 1942b). However, with the exception of secretin which initiates the flow of pancreatic juice, the several gastrointestinal hormones recognized in mammals (Grossman, 1950) have not been identified. Bayliss and Starling (1903) isolated secretin from fish tissues and Babkin (1929) studied its effects, as well as those of many other materials, on pancreatic secretion of the skate, where pancreatic regulation was found to be purely humoral (Babkin, 1931). Babkin *et al.* (1935) denied that gastric secretion in elasmobranchs is under humoral control (see also Chapter III).

2. Renin

The presence of renin in the kidneys of freshwater teleosts and its absence in both marine teleosts and elasmobranchs has been described

(Friedman *et al.*, 1942). Renin production appears to be an adaptive reaction which insures a sufficiently high blood pressure for glomerular filtration. The absence of the renin-hypertensin mechanism in saltwater fishes may be associated with their reduced glomerular activity. In marine fishes body fluids are either hypotonic (teleosts) or approach an isotonicity with sea water (elasmobranchs) and the glomerulus is not called upon to remove large quantities of water (Chap. IV). Consequently a high blood pressure within the glomerulus is not as critical in marine fish as it is in freshwater fish or mammals.

3. Corpuscles of Stannius

The corpuscles of Stannius have been investigated repeatedly but their function is still unexplained. These structures, usually paired, develop as bud-like evagination(s) from the wall of the pronephric duct anterior to the opisthonephros (Holostei) or in the posterior region of the opisthonephros (Teleostei). Corpuscles of Stannius have not been located in other groups and seem to have evolved quite suddenly between Chrondrostei and Holostei. Garrett (1942) discusses the phylogeny and suggests a homology with the Müllerian ducts of the Elasmobranchii and Chondrostei. This might imply a role in the physiology of reproduction. It is agreed that these bodies have the appearance of endocrine tissue, but, contrary to earlier speculation, they are now known to be unrelated to the interrenals (Garrett, 1942; Callamand, 1943). The European eel, *Anguilla anguilla*, survives for a long time after removal of the corpuscles of Stannius (Vincent, 1898). No evidence of an interrelationship between pituitary and corpuscles of Stannius has been found with hypophysectomized *Fundulus* (Pickford, 1953a) or *Astyanax* under "stress" (Rasquin and Rosenbloom, 1954).

4. Pineal Organ

The pineal organ of fishes is a recognized photoreceptor (Breder and Rasquin, 1950), and present indications are that, at least in one species, this structure should also be listed among the endocrine tissues (Pflugfelder, 1953, 1954). Destruction of the pineal organ of the guppy, *Lebistes*, is followed by reduced growth rate, skeletal abnormalities, and a marked stimulation of both pituitary and thyroid glands. Increased activity was also noted in the Corpuscles of Stannius and in remnants of pronephric tissue. It is suggested that the action of the pineal secretions is mediated through the pituitary and thyroid glands.

III. GROWTH, DEVELOPMENT, AND METAMORPHOSIS

A. Pituitary Growth Hormone

Pickford (1953a, 1954a) has established the presence of pituitary growth hormone and studied its physiological effects in *Fundulus*. The hypophysectomized fish do not increase in size or add new circuli to their scales or show additional growth of otoliths. Injections of either purified fish or beef pituitary growth hormone restores growth. The fish growth hormone preparation used was less effective than beef growth hormone, perhaps owing to the total absence of thyrotropin which was readily detected in the latter. Other effects of hypophysectomy were not corrected by purified fish growth hormone. Gonads and thyroid remained quiescent; the anemia was not alleviated; the liver did not show the decrease in size which normally occurs in spring; and renal calculi did not disappear from the kidney. Purified mammalian growth hormone also promotes increased growth in brown trout (Swift, 1954). The existence of a specific growth hormone in fish can no longer be questioned.

Factors controlling production of somatotropin have not been investigated although seasonal effects on the pituitary and on growth have been recognized. In addition, the varying demands for other pituitary hormones may be expected to influence the output of somatotropin. Marked decrease in growth of *Astyanax* was noted when ACTH production was stimulated by "stress" (Rasquin and Rosenbloom, 1954).

B. Thyroid Hormone and Growth

It has been argued that thyroid hormone has morphogenetic rather than metabolic effects in fishes. Thus, thyroid treatment stimulates growth differentially during development so that proportions of fins, body wall, weight, and length are altered, while the deposition of guanine in the skin of salmonids (Fontaine, 1953a) and the rate of scale formation in sturgeons, *Acipenser*, is accelerated (Gerbilsky and Saks, 1947). In addition, growth and morphogenetic processes are slowed down when thiourea or other antithyroid drugs are used (Dales and Hoar, 1954).

It is not likely that thyroid hormone promotes growth in a comparable manner to pituitary growth hormone. In fact, Pickford (1954b) did not find any growth acceleration in hypophysectomized killifish treated with thyrotropin although the thyroid gland was strongly stimulated at higher dosages. Again, La Roche and Leblond (1954) observed no change in growth of Atlantic salmon, *Salmo salar*, when the thyroid was destroyed by radio-iodine. It seems likely that thyroid hormone affects

growth in association with somatotropin. When present in excess, thyroxine may markedly accelerate some chemical process and result in changes in rates of relative growth. Processes responsible for the development of epidermal structures seem most susceptible to thyroid stimulation.

C. Metamorphosis

Many fishes show a true metamorphosis during their development (e.g. lamprey [*Petromyzon*], eel, flounder [*Pleuronectes*]) while others experience a physiological transformation initiating a period of more rapid growth (the parr-smolt transformation in salmonids). There is general agreement that thyroid activity is marked during such periods of morphological and physiological change. In the case of the parr-smolt transformation treatment with thyroid preparations accelerates while antithyroid drugs inhibit certain aspects of the change such as the altering rates of relative growth and the increasing guanine deposition (Robertson, 1949; La Roche and Leblond, 1952; Fontaine, 1953a; Olivereau, 1954). However, it is by no means certain that thyroid hormone is responsible for the transformation. In fact, attempts to induce increased salinity tolerance, which is characteristic of the transformation, were unsuccessful with juvenile coho salmon (unpublished data) treated with thyroxine. Smith (1956) has reported that somatotropin in low concentrations increases salinity tolerance of brown trout fry or parr, while other hormones either produced no effect whatever or, like thyroxine, gave variable effects in high concentrations. The pituitary may control the parr-smolt transformation through its effects on both growth and the thyroid.

Several fishes, like amphibians, may assume a semi-terrestrial existence. The endocrinology of this metamorphosis or transition from aquatic to terrestrial life was studied by Harms (1929). The gobies, *Boleophthalmus* and *Periophthalmus,* and the blenny, *Salarias,* showed an enlargement of the thyroid at the time of migration and Harms was able to induce the change prematurely by the use of thyroid materials. More strikingly, the blenny, *Blennius ocellatus,* which normally spends its entire life in water, assumed an amphibious existence following thyroid treatment and would sometimes breathe air for eight hours.

Attempts to induce metamorphosis of ammocoetes with thyroid substance have been unsuccessful (Leach, 1946) and, although there is histological evidence of thyroid activity in metamorphosing eels and flounders, the changes have not been induced prematurely by thyroid material. In fact, the metamorphogenetic changes seem to be initiated prior to the increased thyroid activity while this activity, as judged from

the histology of the organ, is often no greater than that observed at sexual maturation (Hoar, 1951). It is, therefore, likely that these growth changes are initiated in the pituitary and that thyroid hormone is essential for their normal expression.

IV. METABOLISM

It may seem presumptuous to discuss the interrelationship of hormones and metabolism in fishes while information of fish hormones is fragmentary and knowledge of their intermediary metabolism almost nonexistent. No well-established series of intermediary metabolic steps can be described with certainty and the suggested relations are usually based on inferences from comparable effects with mammals. However, there are so many tantalizing and baffling problems in the metabolism of fishes that this section may not be out of place as a stimulant to research.

A. Oxygen Consumption

Studies of the relationship between thyroid hormone and oxygen consumption have given conflicting results. Although the matter has not been critically examined in cyclostomes, Leach's (1946) work on the lamprey failed to show any relationship between thyroid activity and oxygen consumption. In elasmobranchs it is almost certain that the thyroid has little or no effect on respiration. Matty (1954) has shown that surgical thyroidectomy has no effect on the oxygen consumption of the dogfish, although extracts of dogfish thyroid promote increased oxygen uptake in rats. The situation is less clear in teleosts. Earlier investigators agreed that the teleost thyroid was also without calorigenic action (Drexler and Issekutz, 1935; Etkin *et al.*, 1940). At present, however, this conclusion cannot be accepted without qualification. Smith and Matthews (1948) have reported a rise in oxygen consumption of white grunts (*Bathystoma*) injected with extracts of the thyroids of Bermuda parrot fish (*Sparisoma*) and Müller (1953) claims that both thyroxine and thyroid stimulation cause an increase in the oxygen consumption of goldfish.

Most investigators, but not all, have found that antithyroid materials such as thiourea decrease the demands for oxygen (Fontaine, 1953a). Several investigators have taken advantage of thiourea treatment in counteracting anoxia when moving hatchery fish. Measurements have indicated a decreased oxygen consumption by the thiourea-treated fish. Chambers (1953) summarizes the literature. These findings should be interpreted with caution, however, since thiourea is a strong antioxidant

and the reduced oxygen consumption might be due to this property rather than lack of thyroid hormone.

At present it is impossible to decide whether or not thyroid hormone plays a part in the control of oxygen consumption. Investigations are hampered by technical difficulties. Individual variations are marked and fish are so readily disturbed by handling that it is difficult to obtain a reliable base line. It must be remembered, however, that drugs such as dinitrophenol and hormones such as testosterone produce readily measurable changes in oxygen consumption under experimental conditions which give inconclusive results with thyroxine. These findings are particularly baffling in view of the increased activity which often follows thyroxine treatment (Hoar *et al.*, 1955). Until we understand where these vital iodine-containing molecules fit into the metabolic chain it is not likely that the riddle will be solved.

The effect of adrenaline on oxygen consumption of fish is likewise inadequately understood. Smith and Matthews (1942) found decreased oxygen consumption when *Girella nigricans* was injected intraperitoneally with adrenaline contrary to expectations based on mammalian physiology. Activity induced by handling and injection of saline produced a rise but the same activity associated with adrenaline injection produced a fall in oxygen consumption. These findings are puzzling since adrenaline injection produces as prompt and as marked a rise in the blood sugar level of fish as of mammals (McCormick and Macleod, 1925).

Gonadal hormones stimulate fish to greater activity and induce a general increase in metabolism. Increased oxygen consumption has been described in sexually mature guppies (*Lebistes*), in juvenile salmon and goldfish immersed in methyl testosterone or stilbestrol, in goldfish fed on beef testes, and in goldfish injected with gonadotropin. The latter response was abolished by gonadectomy and must depend on gonadal hormones. The greater oxygen consumption probably results from the increased general activity induced by the gonadal steroids (Hoar *et al.*, 1955).

B. Carbohydrate Metabolism

Insulin plays a key role among the hormones involved in carbohydrate metabolism. In mammals, this hormone facilitates the conversion of glucose to glycogen or to fatty acids. In addition it may control movement of glucose across membranes or oxidation of carbohydrates. The precise mechanism is by no means clear (Stadie, 1954) but it is well known that lack of insulin produces marked hyperglycemia and excess insulin hypoglycemia. Pituitary growth hormone and adrenal corticoids

oppose the action of insulin perhaps by interfering with the hexokinase mechanism (Bishop, 1954).

The almost complete segregation of the islet tissue of teleosts made possible the first unequivocal demonstration that insulin is produced by this organ (McCormick and Macleod, 1925). Removal of the islets produced a marked hyperglycemia in the gray sculpin. Insulin prepared from fish tissues produces the expected fall in blood sugar when injected into mammals (McCormick and Noble, 1924). Hypoglycemia is also characteristic of fish injected with insulin but the effect is somewhat variable in different species and always less marked than expected from mammalian work (Gray and Hall, 1930; Bruun and Hemmingsen, 1938).

Cavallero (1953), in a recent physiological study of *Scorpaena scropha*, found evidence of glucagon as well as insulin in the islet tissue. Extracts of the islets of this fish produce an abrupt fall in the blood sugar level of the normal or diabetic rat (insulin effect). The glucagon effect is evident, however, after the destruction of insulin by alkali, when extracts produce an increase in blood sugar lasting for 60 to 180 minutes. Cobalt chloride (which in mammals affects alpha cells) destroys the glucagon action thus indicating a parallel mechanism in fish and in mammals. Although Weitzel *et al.* (1953) were unable to demonstrate the hyperglycemic (glucagon) effect of fish islets with rabbits as test animals, Cavallero's (1953) results seems to demonstrate conclusively that the alpha (glucagon-producing) and beta (insulin-producing) cells of teleosts and mammals elaborate similar or identical hormones.

A number of investigators have shown that both in elasmobranchs and teleosts alloxan treatment leads to degenerative changes in the beta cells, similar to but not always identical with those observed in mammals (Grosso, 1950; Clausen, 1953; Pallot and Schätzle, 1953a). Succinic dehydrogenase and cytochrome oxidase have been studied in relation to the necrosis of beta cells produced in alloxan diabetes of the toadfish, *Opsanus* (Lazarow and Cooperstein, 1951).

Hypophysectomy (anterior lobe ablation) leads to a fall in the blood sugar of the dogfish (Abramowitz *et al.*, 1940) and a progressive decline in the glycogen reserves of the fasting eel (Hatey 1951a, b). In mammals the pituitary has been shown to be essential for the transformation of endogenous fat and protein to carbohydrate (neoglycogenesis). This diabetogenic action of the pituitary growth hormone (Bishop, 1954) is evidently present in fish as in mammals. In support of this conclusion Hatey's investigations of the hypophysectomized eel showed that the ability to store glycogen from injected glucose was unimpaired. Pickford (1953a) likewise found abundant glycogen and fat in livers of hypo-

physectomized but feeding *Fundulus*. These contributions show that in fish as in mammals the pituitary is essential for neoglycogenesis.

Hartman *et al.* (1944) in their study of the metabolism of the interrenalectomized skate established a relationship between the interrenal and carbohydrate metabolism. In mammals the glucocorticoids not only antagonize insulin but also promote gluconeogenesis. These workers found evidence of the latter relationship in the extremely low glycogen values of the interrenalectomized skates. The blood sugar level showed little change, so an anti-insulin effect was not demonstrated by these experiments.

There is some circumstantial evidence that thyroid hormone is involved in carbohydrate metabolism. The liver glycogen reserves of the young salmon are relatively low during the smolt transformation when the thyroid gland is active. Thiourea also causes depletion of liver glycogen and in various species of fish produces different effects on liver size (Chambers, 1953). These findings suggest that thyroid hormone is directly or indirectly concerned with glycolysis or glycogenesis but there is not yet sufficient information to speculate on the mechanism.

Fontaine and Hatey (1953) discuss the redistribution of carbohydrate stores which occurs in the female salmon at sexual maturity (See also Chap. VII).

C. Protein Metabolism

Pituitary growth hormone must selectively facilitate nitrogen retention and protein synthesis. This would seem to be self-evident although measurements of nitrogen balance have not yet been made.

Corticosteroids promote gluconeogenesis in mammals with a negative nitrogen balance. Hartman *et al.* (1944) found evidence of this gluconeogenetic action in studies of the interrenalectomized skate. Rasquin and Rosenbloom (1954) found excessive protein catabolism in association with endocrine imbalance produced in *Astyanax* maintained under conditions which produced an initial stimulation of the interrenal followed by atrophy. In these fish protein was evidently being used as a source of energy while fat accumulated in the tissues.

Thyroid hormone is related either directly or indirectly to growth and may cause protein anabolism. It certainly promotes deposition of guanine in the skin of salmonids and must in some way be related to the metabolism of nucleoproteins. Small physiological doses of thyroid hormone produce an anabolic effect in mammals while larger doses promote catabolism (Rupp *et al.*, 1949). In view of the effects of thyroxine on morphogenetic processes, nitrogen retention might also be expected in fishes.

Ammonia excretion of normal and thyroxine-treated goldfish has been studied in this laboratory. The amount of ammonia, as well as the total nitrogen values, increase when goldfish are treated for from one to three weeks with thyroxine and fed on either high- or low-protein diets. Determinations were made on the ambient water of fasting animals (16 to 40 hours without food) maintained undisturbed in darkness. The increased values may be caused by excessive dosage, although comparable but even higher values were obtained following treatment with methyl testosterone or stilbestrol. It is suggested that these higher nitrogen excretion values are due to the metabolism of protein in fasting fish stimulated to excessive activity by the hormone treatment (Hoar *et al.*, 1955). It is of interest that changes in ammonia output are readily detected in thyroxine-treated fish, although oxygen consumption is apparently unaltered when measured by the usual techniques. With the gonadal steroids variations in nitrogen excretion and oxygen consumption are both readily detected with routine methods of determination.

D. Fat Metabolism

Fontaine and Callamand (1953) have discussed the relation of the endocrines to liver fat and indicated the fragmentary nature of our knowledge of the endocrinology of fat metabolism. Pickford (1953a) found that hypophysectomy prevented the springtime reduction in liver size which occurs in normal male *Fundulus*. This does not seem to be due to a deficiency of growth hormone (Pickford, 1953b) but may result from lack of thyroid or gonadal hormone following hypophysectomy. Several workers have described a fall in liver stores in association with a seasonal increase in thyroid activity (Fontaine and Callamand, 1953). It is also probable that adrenocorticoids favor accumulation of fats in fish as in mammals. In this connection, Rasquin and Rosenbloom (1954) describe an accumulation of fat in association with loss of protein in *Astyanax* under "stress." The data are suggestive although by no means conclusive, since the whole endocrine system was involved. Fontaine and Callamand (1953) summarize other suggestive data for an interrelation between adrenal corticoids and fat metabolism as well as for an insulin action.

La Grutta (1950) estimated liver lipid in *Scorpaena scrofa* treated with alloxan or with alloxan followed by insulin. With alloxan alone, lipids increased from 14.2 g. % wet weight to 25.3 g. % but were normal (14.6 g. %) when insulin was also injected. Thus, in teleosts, as in rabbits, insulin prevents hepatic steatosis following alloxan. Steatosis is therefore correlated with changes in carbohydrate metabolism

induced by alloxan. Insulin therapy prolongs the life of alloxan-treated fish even up to 20 days.

Several writers have described the fat accumulation which accompanies sexual maturation (Chap. VII). Pallot *et al.* (1946) describe the development of a special zone in the pancreatic islets and suggest that it may be in some way associated with yolk formation. In many species a marked lipid storage occurs in muscles or liver prior to migration and there may be a redistribution of fats as the gonads (particularly the ovaries) develop. The endocrine glands are doubtless involved but the mechanisms have not been elucidated.

E. Water Metabolism

The hormones of the neurohypophysis, produced in the neurosecretory centers of the hypothalamus (Zetler and Hild, 1954), regulate water metabolism in various ways in the different classes of vertebrates. In amphibians water retention is effected through the control of integumentary absorption. The effective agent may be identical with oxytocin (Heller, 1950; Ewer, 1952). In amphibians and in reptiles the renal excretion of water is mediated by glomerular antidiuresis (Sawyer and Sawyer, 1952). This response is associated with oxytocin in frogs and with vasopressin in toads; while the purely aquatic African clawed toad (*Xenopus*) has no such mechanism (Ewer, 1952). In mammals resorption of water is regulated by vasopressin acting on a renal tubular mechanism.

Both oxytocic and vasopressor principles have been found in the pituitaries of cyclostomes (Lanzing, 1954), and teleosts (Heller, 1950; Lazo-Wasem and Weisel, 1952), but the elasmobranch neurohypophysis contains little or none of these (Heller, 1945; Waring and Landgrebe, 1950).

The pituitary regulation of water metabolism in elasmobranchs has not been investigated. Although a water balance factor with a weak effect on amphibians has been described (Heller, 1945), negative results are to be expected since the elasmobranchs as a group have attained osmotic neutrality through the retention of urea (Chap. IV), and their water balance problem must be relatively small.

All teleosts, on the other hand, maintain an osmotic gradient and it is surprising that investigations have given negative results. The factor affecting amphibian water balance has been looked for both in freshwater and marine species (Boyd and Dingwall, 1939; Fontaine and Raffy, 1950; Callamand *et al.*, 1951). The renal regulation of water excretion, however, has been studied little. Burgess *et al.* (1933) report that vasopressin has no effect on urine flow in the catfish, *Ameiurus*, but since

this freshwater species is in a state of natural diuresis the mechanism for eliciting glomerular antidiuresis may be absent, as in the aquatic amphibian, *Xenopus*. Marine species, in which the conservation of water is of paramount importance, have not yet been studied.

Osmotic regulation has been studied in the hypophysectomized eel and in *Fundulus* with conflicting results. After hypophysectomy, elvers of the European eel, *Anguilla anguilla*, which normally seek fresh water, become less markedly halophobic (Vilter, 1946). Callamand *et al.* (1951) found no osmotic difficulties with hypophysectomized adult eels either in fresh or salt water. Pickford (1953a) successfully maintained hypophysectomized *Fundulus* in sea water, although calculi accumulated in the kidneys. The animals died when transferred to fresh water.

There is some evidence that thyroid hormone may be involved in the elimination of water. When marine teleosts are placed in dilute salt solutions, making an increased output of water imperative, the thyroid is markedly stimulated. The effect is not obtained in the reverse situation where water output is decreased (Olivereau, 1954). Experimentally, marine fish survive longer in fresh water when treated with thyroxine or TSH (Fontaine, 1956) and the euryhaline *Fundulus heteroclitus* shows greater thyroid activity in hypotonic than in hypertonic media (Gorbman and Berg, 1955). Thus, the demands on the thyroid mechanism of some fish are greatly increased by a low salinity environment. However, it has not yet been determined whether this demand is created by a requirement for hormone used in eliminating osmotic water, or by the difficulties which some fish experience in elaborating thyroid hormone when the environment is relatively low in iodine. Whatever the explanation, it is indicated that some teleosts may be restricted in their ability to grow and survive in certain environments by the limitations of their thyroid gland. The anadromous smelt, *Osmerus mordax*, when "landlocked" completes its entire life cycle in fresh water and reaches a size similar to that of its sea-going counterpart. Thyroids are more active in "landlocked" individuals but seem capable of meeting the demands. The alewife, *Pomolobus pseudoharengus*, on the other hand, when "landlocked" shows an excessively stimulated or exhausted thyroid gland. Individuals remain small and show a spectacular mortality at the time of reproduction, when there are probably extra demands on the thyroid mechanism (Hoar, 1952).

F. Mineral Metabolism

Fishes are able to control mineral metabolism within narrow limits but there is scarcely any information concerning the role of the endocrines in this regulation. Fontaine (1953b, 1956) has reviewed the available data.

Hypophysectomy does not disturb the chloride balance of the eel (Callamand *et al.*, 1951) but abolishes the ability of the euryhaline *Fundulus heteroclitus* to survive in fresh water or diluted sea water (Pickford, 1953a; Burden, 1956). Death of hypophysectomized *Fundulus* in fresh water is associated with a marked decline in serum chloride and an atrophy of the mucous cells of the gills. Chloride secreting cells show no histological changes. Injections of known pituitary hormones, thyroxine or deoxycorticosterone acetate failed to prolong the lives of these hypophysectomized fish. Survival time was, however, increased by injections of *Fundulus* pituitary brei. Pituitary brei of the fresh water perch (*Perca flavescens*) was less effective than that of *Fundulus* while that of the pollock (*Pollachius virens*), a marine fish, was ineffective. It is concluded that the pituitary of *Fundulus heteroclitus* secretes a factor(s) which regulates its salt balance in fresh water. The factor is apparently lacking in the stenohaline marine fish, *P. virens*, but may be present in the freshwater species, *P. flavescens*.

There is now strong evidence for an endocrine control of calcium metabolism in fish but details of the mechanism are not yet clear. A marked decline in serum calcium follows hypophysectomy of the eel (Fontaine, 1956). The formation of carbonate-apatite renal concretions in hypophysectomized *Fundulus* indicates a disturbance of calcium metabolism. Rasquin and Rosenbloom (1954) found that faulty bone development and formation of renal calculi can be induced by general endocrine imbalance involving hypertrophy of the ultimobranchial gland in *Astyanax*. Skeletal abnormalities have also been reported in the pinealectomized *Lebistes* (Pflugfelder, 1953). The endocrine control of calcium metabolism in fishes is a tantalizing research problem.

In higher vertebrates the adrenal corticoids are indispensable for the regulation of salt metabolism. However, several investigators have failed to show any relationship between electrolyte balance and interrenal hormones of fish (Fontaine, 1956). Brown trout (*Salmo trutta*) injected with cortical steroids show a rise in muscle sodium and a fall in muscle potassium, serum sodium, and serum potassium (Chester Jones, 1956). These changes are expected on the basis of mammalian physiology but may be pharmacological rather than physiological. Interrenalectomy produces no significant changes in the plasma sodium or potassium of the skate (Hartman *et al.*, 1944).

Several workers find that thyroid hormone, on the other hand, affects the chloride balance of euryhaline and anadromous fish (Fontaine and Koch, 1950). Some of the most interesting experiments were performed by Heuts (1943) and Koch and Heuts (1943) on the euryhaline stickleback, *Pygosteus*. Immature animals are remarkably tolerant to changing

salinity. Salinities ranging up to 35 ‰ produce little alteration in blood chloride. At spawning time, however, there is a marked decrease in salinity tolerance and this is associated with increased activity of the thyroid gland. Experimental confirmation of the theory that thyroid hormone decreases salinity tolerance in sticklebacks was found when fish fed on thyroid gland were unable to withstand salt water and died with accumulation of chloride. Fontaine (1954) believes that thyroid hormone is important in maintenance of salt as well as water balance of fish and has, in several papers, discussed circumstantial evidence based on thyroid changes during migration of the anadromous salmon, *Salmo salar*. The significance of osmotic stress in inducing the appetitive behavior of migration has also been discussed (Hoar, 1953).

V. REPRODUCTION [2]

Hormones of the pituitary (gonadotropins) and gonads (androgens, estrogens, perhaps progesterone) are intimately involved in processes which regulate the timing of reproduction and control its physiology. Associated changes in thyroid and interrenal have also been described but the present discussion will be limited to the pituitary and gonads. The literature has recently been reviewed (Dodd, 1955; Hoar, 1955).

A. Gonadotropins

1. Cyclostomes

The effects of hypophysectomy on gonads have not been studied and attempts to demonstrate gonadotropic effects have been made only with mammalian preparations. Although the findings have not always been in agreement, it is evident that a variety of steroids will induce changes in the secondary sex characters (cloacal swelling) and produce a precocious differentiation of the germinal tissue. The former effect appears consistently with pregnancy urine or mammalian pituitary preparations while the response of the germinal tissue has been regularly obtained only with pregnancy urine. Dodd (1955) points out that the discordant results may be attributed to seasonal variations in responsiveness, or to temperature differences as well as to specificity of compounds. Pregnancy urine contains such a variety of steroids that results obtained with this material are not particularly useful in elucidating controlling mechanisms. The effects may be indirect (de Groot and Duyvené de Wit, 1949) or due to a general pharmacological action. Until hypophysectomized and gonadectomized animals are studied with extracts of cyclostome

[2] I am indebted to J. M. Dodd for unpublished tables and summaries of the literature for this section.

organs or purified preparations, it will not be possible to present a clear picture of the endocrine control of reproduction in the most primitive group of vertebrates.

2. Elasmobranchs

Dodd (1955) has emphasized the endocrinological interest provided by the elasmobranchs as a group. Closely related species may be oviparous or viviparous and all species develop post-ovulatory corpora lutea and possess a Müllerian duct system which by analogy with higher vertebrates might be under endocrine control.

Vivien (1941) demonstrated regression of the gonads following hypophysectomy of the dogfish (*Scyliorhinus canicula*). Although the response did not appear for many weeks the experiments seem to justify the conclusion that the pituitary dominates the gonads. Unpublished experiments by Hisaw and Albert on *Mustelus canis* (viviparous species) show that hypophysectomy suppresses ovulation (restored with pituitary implants) but does not affect the development of corpora lutea or influence the course of gestation, at least during the first three months. Hisaw's findings suggest that in *Mustelus*, a species with well-developed yolk sac placenta, the action of gonadotropins ends with ovulation and the pituitary influence on corpus luteum and gestation must have evolved later (Dodd, 1955). It is probable that species variations will be found among the oviparous and viviparous forms. For example, in *Scyliorhinus*, contrary to *Mustelus*, removal of the anterior lobe did not affect ovulation for a period of at least 10 weeks (Dodd, 1955).

Histological changes have been described both in the anterior and intermediate lobes of the elasmobranch pituitary during sexual maturation and gestation (Carere Comes, 1936; Ranzi, 1936). An increased eosinophilia in the intermediate lobe, an accumulation of basophilic colloid in the tubules of the anterior lobe, and a marked hyperemia were noted. In teleosts, gonadotropic activity is localized in basophils of the middle glandular area (Atz, 1953). The source of pituitary gonadotropins seems to be different in these two classes of vertebrates.

Elasmobranchs have rarely been tested with mammalian gonadotropin but in the two investigations reviewed by Dodd the effect was positive.

3. Teleosts

The gonadotropic activity of the teleost pituitary has been investigated repeatedly since the fish culturist has found it distinctly advantageous to induce early spawning by appropriate manipulation of the endocrines (Fontenele, 1955). A summary of the literature (Hoar, 1951, 1955) shows that the activity of the gonadotropin of the middle glan-

dular area fluctuates seasonally; that there is some evidence of species and group specificity of gonadotropins but that, in general, pituitary preparations from any species of fish or cold-blooded vertebrate have marked gonadotropic action in other fishes. On the other hand, fish are so unresponsive to many mammalian (and bird) preparations that it is generally impractical to use them. The fish culturist or physiologist who wishes to hasten gonad development in fish is advised to use fish pituitaries. However, pregnancy urine and chorionic gonadotropins frequently give positive results but it is difficult to say which of the many factors present in these materials is active.

The mammalian physiologist has been able to separate two different gonadotropic substances. FSH (follicle-stimulating hormone) stimulates development of the ovarian follicle in the female and the seminiferous epithelium in the male; LH (luteinizing hormone) or ICSH (interstitial cell-stimulating hormone) is involved in corpus luteum formation in the female and interstitial cell development in the male. All these functions have been induced in fish by fish pituitary extracts or eliminated by hypophysectomy.

In a cytological investigation of the pituitary of *Astyanax*, Atz (1953) found only one type of periodic acid- Schiff-positive gonadotrophe. The activation of these gonadotropic cells or pituitary injection was associated with a proliferation of the follicular epithelium of the ovary and the appearance of cytoplasmic granules which blackened with osmic acid (Rasquin, 1951). In males, the increased secretory activity of the sperm duct epithelium distended the duct and testicular lobules with fluid.

Experiments of Witschi (1955) indicate that the pituitary of bony fishes is low in FSH (tested by vaginal cornification in rats) but contains normal amounts of LH (tested by the feather reaction in weaver finches) as compared with mammals. It is by no means certain, however, that the same biochemical materials are operating in groups as widely separated as mammals and fish. In fact the refractoriness of fish to mammalian gonadotropins indicates real differences. Pickford (unpublished data) finds that the regressed testes of hypophysectomized *Fundulus* are stimulated by administration of androgens (as in mammals) or by repeated injections of mammalian LH, but FSH (not completely free of LH activity) is relatively ineffective. Carlisle (1954) found that sperm release in male sharks, and the emission of genital products in ascidians, can be elicited equally well with a number of mammalian gonadotropins, including prolactin. It is possible, as Carlisle has suggested, that the several mammalian gonadotropins may have evolved from a primitive hormone and that there is little differentiation of these factors in lower vertebrates. Until the problem can be investi-

gated with precise chemical methods the nature of the specificity cannot be established.

In addition to the gonads, several other target organs associated with reproduction may be regulated by pituitary hormones. Although no certain function has been attributed to prolactin in fish, there is suggestive evidence for its presence in fish pituitaries (Leblond and Noble, 1937). The spawning reflex of *Fundulus* is elicited by posterior lobe hormones (oxytocin and vasopressin), or by pituitary fractions with posterior lobe activity (Pickford, 1952; Wilhelmi *et al.*, 1955). Test animals may be intact, hypophysectomized, or castrated, and the response can be elicited equally well in either sex.

B. Gonadal Hormones

It is assumed that hormones are elaborated by the gonads of all fishes although gonadectomies seem not to have been performed in cyclostomes and elasmobranchs. This operation has frequently been performed on different teleosts (also on *Amia calva*) and has resulted in disappearance or complete absence of secondary sexual characters and behavior.

The chemical nature and site of formation of the gonad hormones is uncertain. Extracts of fish gonads assayed biologically or tested chemically indicate the presence of androgen(s) in males and estrogen(s) in females but the true identity of the steroids has not been established (Hoar, 1955). The presence of highly developed post-ovulatory corpora lutea in the elasmobranchs suggests the formation of progesterone or related material but this is entirely speculation. In fact the endocrinology of pregnancy and gestation in fish has rarely been investigated.

A considerable volume of literature is available on the physiological effects of androgens and estrogens on fish. Gonad development, secondary sex characters, and behavior can be readily modified by a variety of naturally occurring or synthetic steroids. The results, however, are not always those which would be expected on the basis of mammalian physiology (Tavolga, 1949, and reviews cited). The Poeciliidae have been used most frequently as test animals. In this group, the intromittent anal fin or gonopodium of the male forms a well-marked secondary sex character which can be readily modified by gonadal steroids. *Gambusia, Lebistes, Platypoecilus, Xiphophorus, Oryzias,* and *Mollienesia* have been studied. Other families have received less attention. However, *Carassius, Fundulus, Salmo, Oncorhynchus, Acheilognathus, Phoxinus, Misgurnus, Ctenopharyngodon, Scyliorhinus,* and probably other species have been injected or otherwise treated with gonadal steroids. The numerous references are not given here since detailed reviews of the literature are available (Dodd, 1955; Hoar, 1951, 1955).

Testosterone propionate has been most frequently used to produce

androgenic effects, although ethynyl testosterone (pregneninolone), methyl testosterone, and testosterone acetate have been tried. Methyl testosterone is two to three times as active as pregneninolone in the gonopodial test with female *Gambusia* (Turner, 1942) but, in general, the relative activities of the different androgens have not been established. Estradiol or estradiol benzoate have been the preferred estrogens. Estrone, estriol, and stilbestrol have also been shown to be effective.

The androgenic action of testosterone compounds is pronounced. In the Poeciliidae gonopodium formation is readily induced in juveniles, in females, or in castrates. The fin formed, however, is sometimes atypical and other secondary sex characters, such as pigment in male *Lebistes*, may not appear; male behavior is sometimes induced or exaggerated; the gonads are altered; ovaries show regression, with resorption of yolk and embryos in viviparous species, or they may be transformed to testes; testes may also regress with prolonged treatment probably due to the inhibitory action of the androgen on the pituitary. Likewise in the elasmobranch, *Scyliorhinus canicula*, testosterone propionate has a strongly androgenic action producing hypertrophy of the anterior part of the Wolffian duct in genetic females (Thiebold, 1954).

Estrogenic effects have been most frequently observed in teleost gonads. In the testes, there may be exhaustion, degeneration, or transformation to ovaries. Ovarian development is accelerated. However, the reverse effects have also been observed in both ovaries and testes (Tavolga, 1949; Egami, 1954a). Inconsistencies may be attributed to dosage, variable physiological state of the target organs, and interaction of other endocrine glands. In the elasmobranch, *Scyliorhinus*, estradiol benzoate has an estrogenic action causing differentiation of nidamental glands, retention of Müller's canal, and the formation of ovotestes in genetic males (Thiebold, 1954). Chieffi (1954) has investigated the effects of several steroids on sexual development in this species.

In many of these studies it is evident that the target organs (secondary sexual characters) are not responsive at all times. During certain seasons and at different ages gonadal steroids may be quite inactive. Young salmon, for example, show marked metabolic effects but no evidence of sexual behavior or secondary sexual characters when treated with methyl testosterone or stilbestrol (Hoar, 1955). Likewise, the effects on the gonopod of the Poeciliidae varies with the stage of development (Hopper, 1949).

VI. SEASONAL CYCLES OF ENDOCRINE ACTIVITY

Recurring cycles of growth, migration, and reproduction are associated with cyclical changes in the activity of the endocrine glands. Sometimes these rhythms occur under constant environmental conditions but

in the majority of cases they follow seasonal or diurnal cycles. Most animals experience regular changes in illumination and endocrine activity of fish is frequently modified by light. In addition, activities of poikilothermous animals are dependent on environmental temperature and this variable may well affect the endocrine production along with other processes. Osmotic differences, salinity, odors, or tastes may also have an effect (Hoar, 1951, 1955).

The pituitary gland is the first link between the receptor organs and the endocrine system. Environmental effects on other endocrine glands are probably mediated through the pituitary. Temperature might act directly on gonads and thyroid, or the salinity of the environment might modify the ionic and osmotic content of the blood and thus stimulate the thyroid. However, the pituitary produces the tropic hormones and changes in the thyroid or gonads do not normally occur in the absence of a pituitary. It seems safer to conclude that the external environment mediates its effect on the endocrine system through the pituitary. The anatomical pathways from the peripheral sense organs to the pituitary have been carefully described (Bretschneider and Duyvené de Wit, 1947; Green, 1951; von Metuzāls, 1952).

A. Gonadotropic Activity

Seasonal changes in gonadotropic activity have been established by differential counts of pituitary cell types (Scruggs, 1951; Olivereau, 1954) and by testing the efficacy of injecting pituitaries or pituitary extracts into sexually immature fish. Gonadotropic activity is evidently low just after spawning but may be high for several months prior to breeding in seasonal spawners (Gerbilsky, 1940).

Both light and temperature have been shown to control reproduction. The interrelationships of these factors have been carefully studied by B. Baggerman working in Baerend's laboratory (Groningen). In her experiments, as yet unpublished, nest building by the male stickleback was chosen as the criterion for full reproductive development. Sticklebacks transferred from the sea in December (temperature 6° C. and daylight about 8 hours) to the laboratory at 20° C. and 16 hours illumination built nests within 4 weeks. Six-month-old fish (hatched in April and May), if brought into the laboratory in October and held at 20° C. with 16 hours illumination, will mature in 50 days but fail to mature in 8 months with only 8 hours illumination. The acceleration produced by 16 hours illumination was markedly delayed at temperatures below 20° C.

The importance of "photoperiod" in controlling sexual maturation of

Gasterosteus has thus been established in accordance with earlier experiments by Vanden Eeckhoudt (1947) and contrary to Craig-Bennett (1931). The relative importance of light and temperature probably varies in different species (Medlen, 1951). Bullough's (1940) study of *Phoxinus laevis* showed an inherent rhythm in endocrine activity which could be modified by temperature or light but persisted even under constant conditions. In some cases the relative importance of these factors appears to vary in the two sexes of the same species (Merriman and Schedl, 1941).

Diurnal variations in illumination are also of significance in regulating endocrine activity. *Oryzias latipes* normally spawns a few eggs each morning but this rhythm, probably controlled through the pituitary, can be readily modified by changing the photoperiod and becomes irregular under constant illumination (Egami, 1954c). Constant darkness may so greatly disturb *Astyanax* that a condition of "stress" with hypertrophy and eventual atrophy of the endocrine system is evident.

Control of pituitary activity by chemical changes in the internal environment is well established. Increased production of gonadal hormones decreases the output of gonadotropins and the same sort of reciprocal interrelation has been described in other organs. It is possible then that changes in the chemical nature of the external environment may also affect the activity of the pituitary. Those materials which can penetrate gill membranes will reach the pituitary readily. Bretschneider and Duyvené de Wit (1947) have shown that a variety of steroids will act on the gonads when dissolved in the ambient water. They argue that the effect is indirectly mediated through the pituitary. The basis for a control of the pituitary by the chemical environment is thus indicated but further work will be required to provide examples of its significance in nature.

B. Thyrotropic Activity and Growth Hormone

Seasonal changes in thyroid activity and in growth rate suggest that thyrotropic and pituitary growth hormones are produced in varying amounts at different seasons. Although the matter has not been analyzed it is reasonable to assume that photoperiod and temperature act through the pituitary to control production of somatotropin and thyrotropin as well as gonadotropin.

Several investigators have presented histological evidence of increased thyroid activity in the spring (Hoar, 1939; Barrington and Matty, 1954; Olivereau, 1954). Using epithelial height as an index, Barrington and Matty (1954) found maximum thyroid activity in *Phoxinus* between

February and May. The germ cells matured during this period. The changes appeared to be independent of temperature and were probably associated with the breeding cycle. However, experimental manipulation of either environmental temperature or light (Hoar, unpublished) may modify thyroid activity. The effects seem to vary with the species. Thus, for example, a lowering of the temperature stimulates the thyroids of *Salmo gairdnerii* and *Umbra limi* but depresses thyroid activity in *Fundulus, Phoxinus,* and *Lebistes* (Fortune, 1955; Gorbman, 1955 and unpublished; Olivereau, 1955a, b). In several other fish (*Cyprinus, Tinca, Anguilla, Scyliorhinus*) the thyroid gland is apparently not affected by temperature change (Olivereau, 1955a, b).

Swift (1955) traced seasonal changes in thyroid activity and growth of *Salmo trutta.* The index of thyroid activity was determined by the rate of loss of radio-iodine from the thyroids of living fish. Thyroid activity was found to reach a peak in early July, a period of maximum daylight and high temperature. The rate of growth showed two distinct peaks, one in April and the other from September to November. The decreased growth during the period of maximum thyroid activity in June and July is interesting. Several factors may be involved. Extra food is required to meet the increased metabolic demands produced by high temperatures and relatively less food may thus be available for growth. Again, the thyroid mechanism may itself be indirectly stimulated by some particular metabolic demand (e.g. increased output of osmotic water at higher temperatures) or directly through the action of light and temperature on the pituitary–thyroid mechanism. Increased thyroid hormone may be expected to stimulate swimming and general activity, thus creating extra demands on food resources and restricting the amount available for growth. Present data are inadequate to untangle these relationships.

C. Islets of Langerhans

Seasonal changes in the histology of the islets of Langerhans have been studied. Pallot (1939) examined islet tissue from eight species of teleosts and noted increased numbers of alpha cells with colloid accumulation during the winter. In the spring colloid is released and the number of alpha cells decreases. Changes in the number of beta cells are less evident although they are somewhat more numerous in spring and summer, perhaps associated with seasonal changes in growth and carbohydrate metabolism (Pallot and Schätzle, 1953b).

A marked activity in the islet tissue was also associated with sexual

development (see also p. 266). A special *"zone sexuelle"* forms between the exocrine and insular tissue during the pre-spawning period and disappears abruptly after spawning (Pallot *et al.*, 1946).

D. Environmental Regulation

In general it is evident that the seasonal changes in light and temperature may induce marked activity in the pituitary with a resulting surge of endocrine activity. In some cases (spring spawners, such as minnows and sticklebacks) increasing illumination and rising temperature serve as stimuli (Harrington, 1950); in other cases (fall spawners such as salmon and trout) decreasing illumination and falling temperatures are the operating factors (Hazard and Eddy, 1951). In any case, the organism has evolved in an ever changing environment and its activities are closely regulated by environmental conditions. All species investigated have shown some such interrelationship of seasonal cycles with changes in endocrine activity. The fluctuations are, however, particularly marked in strongly migratory fishes and Fontaine (1954) has speculated on the role of the endocrine glands in the evolution of migration. It is possible that this puzzling behavior has evolved over a long period in association with marked cyclical fluctuations in endocrine activity.

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Chapter VII

THE GONADS AND REPRODUCTION

William S. Hoar

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I. Introduction

Fishes as a group exhibit almost every type of sexual reproductive mechanism found in the animal kingdom and several specializations have appeared which are without parallel elsewhere among vertebrate animals. Cyclostomes are the most primitive of existing vertebrates and show the least range in specialization. Eggs or spermatozoa pass from unspecialized gonads through the coelom, to be fertilized and developed externally. Yet even this simple sequence of events may be associated with complex patterns of migratory and reproductive behavior. Among

the Chondrichthyes internal fertilization is universal and is associated with the production of relatively small numbers of young, either from large eggs protected in horny cases or by viviparity. In the Osteich-

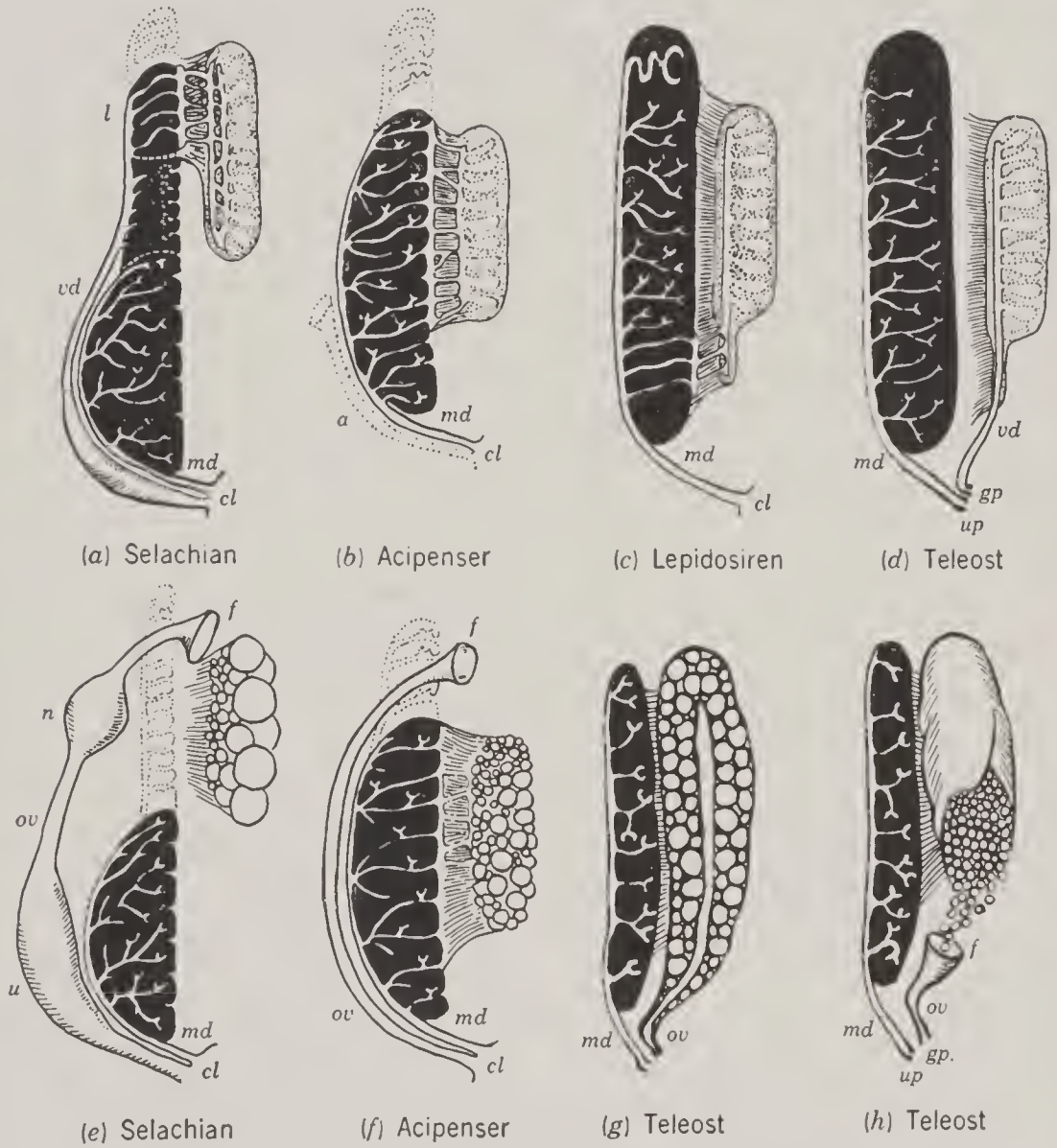

FIG. 1. Representative types of urogenital systems in fishes. Upper series, males; lower series, females; black organs, mesonephros; stippled organs, testes; organs with circles, ovaries; cross-lined, mesentery; stippled lines, vestigial structures; cl, cloaca; f, open funnel of oviduct; gp, genital papilla and pore; l, Leydig's gland; md, mesonephric duct; n, nidamental gland; ov, oviduct; sv, seminal vesicle; u, uterus; up, urinary pore; vd, vas deferens. Redrawn from Portman (1948) with the permission of Dr. A. Portman and B. Schwabe (Basel) and from Goodrich (1930) with permission of Macmillan & Co., Ltd.

thyes, however, the greatest diversity is manifest. The more primitive groups (Dipnoi, Chondrostei, and Holostei) are oviparous, producing numerous eggs which are fertilized externally. The modern and highly successful Teleostei have evolved a spectacular array of reproductive

devices. Viviparity, superfetation, hermaphroditism, extreme sexual dimorphism, and complex breeding behavior involving migration, nesting, brooding, and parental care appear in one or another species of this diversified group of animals.

II. STRUCTURAL RELATIONSHIPS

In the Cyclostomes, eggs and spermatozoa are discharged into the peritoneal cavity and pass to the exterior through genital pores. Gonoducts are characteristic of the other fish-like vertebrates (Fig. 1). Their phylogeny in the different groups is by no means certain. Most workers adhere to the classical description given by Kerr (1901) in which the origin of the male system is traced from the mesonephros and its duct. In less specialized forms (Fig. 1a, b) a well-developed network of the anterior mesonephric tubules convey spermatozoa from the testis to the mesonephric duct. In the more specialized condition there is an enlargement of the posterior element of the network and an atrophy of the remainder to form a single spermatic duct (Fig. 1c, d).

The phylogeny of the female gonoducts is even more problematical. In the Chondrichthyes, Dipnoi, Chondrostei, and *Amia* among the Holostei, ova are discharged into the peritoneal cavity and move into the anteriorly located open end of the Müllerian duct (Fig. 1e, f), while in the Lepidosteoidei and Teleostei the lumen of a hollow ovary is continuous with the oviduct (Fig. 1g). This "cystoarian" condition, as opposed to the "gymnoarian" condition of the groups having naked ovaries, is probably secondary and is formed embryologically by the growth and fusion of peritoneal folds around the gonad. In female Salmonidae, Galaxiidae, Hyodontidae, Notopteridae, Osteoglossidae, and in *Misgurnus* the oviducts degenerate in whole or in part so that ova pass into the peritoneal cavity and thence through pores or funnels, depending on the degree of degeneration, to the exterior (Fig. 1h). In the Anguillidae the loss of gonoducts occurs in both males and females (Goodrich, 1930).

A. Hermaphrodites and the Determination of Sex

Primordial germ cells originate during gastrulation and either migrate or are carried passively by formative processes to the region of the differentiating gonad. Okkelberg (1921) describes these cells in a classical paper on the cyclostome, *Entosphenus wilderi*, and Johnston (1951) has more recently traced their history in the bass, *Micropterus salmoides*.

The gonads of elasmobranchs, like Amphibia and Amniota, have a double origin (D'Ancona, 1950, 1952; Chieffi, 1954). The cortex, originating directly from the coelomic epithelium, is said to produce a femin-

izing action through its inductor material *cortexin*. The medulla, originating from the mesonephric blastema, exerts a masculinizing effect through its inductor *medullarin* (D'Ancona, 1949; Witschi, 1951). Germ cells, migrating into the gonad, are thought to differentiate into spermatogonia or oogonia under the influence of these substances. The chemical nature of the inductors and their relation to the androgens and estrogens of adult vertebrates have not been established.

In cyclostomes and teleosts the entire stroma of the gonad is homologous with the cortex of other vertebrates (D'Ancona, 1952). Inductor substances are presumed to be present although not localized in different areas. D'Ancona (1949) regards this condition as phylogenetically primitive. Two somewhat different situations are recognized among teleosts. In the majority of species, the gonads differentiate from an indifferent state to form about equal numbers of male and female individuals (D'Ancona, 1950; Ashby, 1952; Robertson, 1953). However, the two sexes may differentiate at somewhat different times so that either male or female individuals predominate in samples of embryos at certain stages. This has lead to confusion with descriptions of protogynous or protandrous hermaphrodites in cases where oogenetic or spermatogenetic tissue intermingles with indifferent elements. Such distinctions are probably not justified (D'Ancona, 1950).

The second type of teleost sexual differentiation is observed among the Sparidae and Serranidae. In these two families the gonads show distinct testicular and ovarian zones. Hermaphroditic individuals are common and some species are invariably monoecious and self-fertilizing (van Oordt, 1933). Occasional hermaphroditic individuals have been described in several species of fish but functional hermaphrodites are normal only in the Sparidae and Serranidae. Circumscribed areas of testicular and ovarian tissues may be found in the adult ovary or there may be an intermingling of ovarian follicles with spermatogenetic tissue (van Oordt, 1933; D'Ancona, 1950). Earlier descriptions of hermaphroditism in the cyclostome, *Myxine*, (Dean, 1923, p. 503) have been questioned (Conel, 1917) and functional hermaphroditism is apparently normal in only two families of teleosts.

On the whole, sex determining mechanisms seem labile in fishes and it is perhaps not surprising that sex ratios have been modified experimentally by a variety of procedures. Variations in time of fertilization (Huxley, 1923), rearing temperatures, food supply, and available space (Eberhardt, 1943) have been shown to alter sex ratios. The effects of gonadal steroids on sex differentiation and on the gonads is discussed in Chap. VI. Genetics of sex determination are considered in Vol. II, Chap. X.

B. The Male

1. Structure of the Testis

The ductless testis (cyclostome) is made up of cyst-like units within which the spermatogenic tissue differentiates into nests of spermatozoa which are discharged into the peritoneal cavity when the cyst ruptures (Conel, 1917). When gonoducts are present the cysts of the testis are arranged in lobular or tubular units. Details of the architecture have recently been studied in several teleosts and numerous references will be found in articles by James (1946), Weisel (1949), and Cooper (1952). The testis of the elasmobranch (*Cetorhinus maximus*) has been described by Matthews (1950). References to the earlier literature will be found in Dean's (1923) bibliography.

The lobular units of the teleost testis may be short and tubular or intricately divided spaces with their apices at the center of the organ and their broader ends directed to the periphery. Simple lobules such as those found in the testes of *Gillichthys* project individually from the spermatic duct (Weisel, 1949). In most fish, however, the complex network of elongated and intricately divided lobules and lobular passages is bound together forming a compact organ. Septa and coverings contain much elastic tissue but no muscle. The lobules open into a spermatic duct which may be long and tortuous with a lining of secretory epithelium (Rasquin and Hafter, 1951) or straight and simple with a large, posterior, fan-shaped "seminal vesicle" projecting ventrally (Coujard, 1941a, b; Weisel, 1949).

The lobules contain numerous cysts composed of maturing germ cells varying in appearance with the season and the state of sexual maturity. Within each cyst the cells behave as a unit and are in the same stage of maturity. There may or may not be a seriation of spermatogenesis from cortex to medulla. Turner's (1919) description and illustrations of spermatogenesis in the perch, *Perca flavescens*, are excellent.

2. Secretions of the Sperm Ducts

The genital passages of the elasmobranchs are always specialized to secrete nourishing and protective materials for the sex cells. Borcea's (1906) classical monograph should be consulted for a description of the anatomy and embryology of the parts. The anterior portion of the mesonephros of the male elasmobranch provides not only an efferent duct system between testis and sperm duct but also a mass of small glandular tubules (Leydig's gland). The secretion of these tubules is involved in the formation of the spermatophores and is said to preserve

the vitality of the spermatozoa. The enlarged lower end of the genital duct (vesicula seminalis) is also secretory in nature and completes the formation of spermatophores (Matthews, 1950) or otherwise aggregates sperm for discharge (Parker and Burland, 1909).

The secretory nature of the lining epithelium of the teleost sperm duct and the presence of "seminal vesicles" in some forms (Blennoidea, Gobeiidae) have already been referred to. These teleost "seminal vesicles" do not store sperm and are in no way homologous with the accessory male organs of mammals. Their precise function and that of the secretory epithelia of the genital ducts have not been established. Weisel (1949) suggests that the fluid may keep spermatozoa in an inactive state or nourish them while Rasquin and Hafter (1951) speculate that the secretion is analogous to a combination of mammalian prostatic and seminal vesicle secretions. Coujard (1941a) believes these secretions serve the male blenny in his attendance and care of the eggs perhaps by forming adhesive or lubricating material. The same writer has described another small accessory gland of unknown function in the male goby (Coujard, 1941b).

3. Sexual Maturity and Seasonal Changes in Reproductive Activity

Many male fish attain sexual maturity at an earlier age and smaller size than the females. For example, male Atlantic salmon parr, *Salmo salar,* (about 10 cm. long) while still in fresh water may become sexually mature and engage in spawning with the large (60–70 cm.) sexually mature females returning from the ocean (Jones and King, 1952). Among male fishes this attainment of an early sexual maturity may be associated with a shorter life span (Table I).

TABLE I

Maximum Age Recorded for Different Species of Flat Fish (Heterosomata)[a]

Species	Maximum Age Recorded	
	Males (years)	Females (years)
Platichthys stellatus	5	7
Microstomus pacificus	12	13
Hippoglossus stenolepis	25	35
Parophrys vetulus	15	17
Eopsetta jordani	16	23
Lepidopsetta bilineata	10	13

[a] Data summarized by K. S. Ketchen, Pacific Biological Station (Canada) from several sources.

On a seasonal basis also the male often attains a potential maturity before the female. In the perch or stickleback (*Gasterosteus*), for example, a very short period of quiescence after spawning is followed by a wave of spermatogenic activity resulting in a potential maturity and a testis filled with spermatozoa for several months before the next breeding season. In such cases, functional maturity and breeding do not take place until the secondary sexual characters and behavior appear and coincide in timing with the production of mature eggs by the female (Fig. 2). In other species the testis remains in a state of involution or

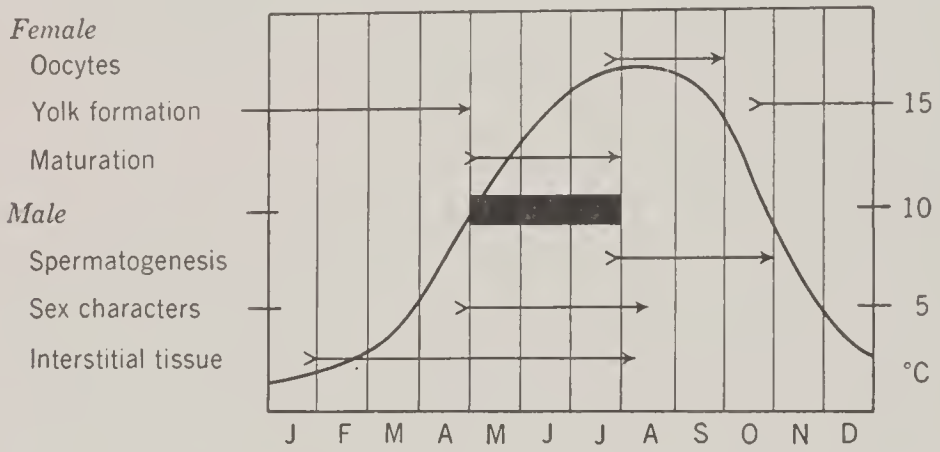

FIG. 2. Seasonal cycle of reproduction in *Gasterosteus*. Data from Craig-Bennett (1931), reproduced from Hoar (1955). (By permission of the Cambridge University Press.)

repose until the approach of the next breeding season (Vivien, 1941). In species which do not show marked seasonal rhythm in reproductive activity the various stages in spermatogenesis may be observed at all times.

Well-marked seasonal cycles of activity have also been described in the male accessory glands of the blenny (Coujard, 1941a) and the goby (Vivien, 1941; Pilati, 1950). Weisel (1949), however, found little seasonal change in the seminal vesicles of *Gillichthys*.

4. Interstitial Tissue and Sertoli Cells

Seasonal variations in the amount of interstitial and connective tissue cells of *Gasterosteus* have been described in detail (Craig-Bennett, 1931). In this species the development of interstitial tissue, probably the source of the testicular androgens, coincides with the appearance of secondary sexual characters and reaches a climax at spawning time. Interstitial cells have been described by several workers in different species of fish. However, they are inconspicuous or absent in testes of many fish, and some of these have marked secondary sexual characters

and complex breeding behavior. The source of testicular hormones and the role of the interstitial tissue in the fish testis is discussed in Chap. VI.

Sertoli cells have been described in both elasmobranchs and teleosts and show cyclical changes in relation to spermatogenesis. Fratini (1953) has studied their cytochemistry in the testis of *Scyliorhinus* and suggests that they play a nutritive and trophic function in sperm development. In the stickleback, Craig-Bennett (1931) noted these cells after sperm discharge but they soon became obliterated by primary spermatocytes and could not be distinguished while spermatogenesis was in progress. Sertoli cells may have an endocrine function (Chap. VI).

C. The Female

1. The Ovary and Egg Production

The ovary is made up of numerous ovarian follicles embedded in connective tissue. The arrangement of the follicles within the supporting tissue varies greatly in different groups. Brock (1878) provided a classical account of the teleost gonad based on an examination of 57 species and distinguished eight different morphological types. More recent descriptions of teleost ovaries are given by Bretschneider and Duyvené de Wit (1947) and Cooper (1952). The ovary of *Myxine* has been carefully studied by Lyngnes (1936) but there are no other recent and detailed accounts of ovarian structure in non-teleost fishes. Dean's (1923) bibliography should be consulted for the older literature.

New batches of ovarian follicles arise periodically from the germinal epithelium. At first each contains a small yolkless egg which is deeply basophilic in fixed and stained material. A quota of these yolkless eggs matures at each breeding season through progressive increase in size, the addition of yolk, and maturation of the nucleus.

Some indication of spawning habits and duration of spawning can be gained by measuring the diameters of the eggs in an ovary. If the spawning period is short, all of the maturing eggs will be of about the same size and this size will be very different from that of the younger follicles. If the spawning period is long or continuous a greater succession of sizes will be found between mature eggs and immature follicles (Hickling and Rutenberg, 1936).

There is considerable variation in the number of eggs produced in different species of fish. As might be expected, viviparous species and species exercising parental care produce fewer eggs than those species which broadcast their eggs and leave them unattended. In oviparous teleosts, egg number increases logarithmically with the size and age of the fish at least until the fish nears the end of its life span when there

may be a definite decline (Raitt, 1932). Even within the same species of fish, however, local stocks may exhibit considerable differences in egg number. Sockeye salmon (*Oncorhynchus nerka*) from Chilko Lake of the Fraser River in Canada produce about 2260 relatively small eggs (5 year average) while sockeye salmon of the same size (564 mm.) from Cultus Lake in the same river system produce 3860 relatively large ones (7 year average). The adaptive significance of such differences is not understood.

Production of large numbers of eggs would seem to confer an evolutionary advantage and mechanisms must be present to restrict this tendency. Svardson (1949) argues that a large egg confers an advantage on the resulting fry and that egg number is restricted by this tendency to produce eggs of larger size. It is true that among teleosts in general species which produce larger eggs, such as the Salmonidae, produce fewer eggs (1500 to 5000 eggs) than species producing smaller eggs such as the Gadidae or Pleuronectidae (100,000 to 2,000,000 eggs). It is also true that in more northern latitudes, where conditions are perhaps more rigorous, the different species of several genera produce larger and fewer eggs (Rass, 1935). Scott (1956) has studied egg size and egg number in trout, *Salmo gairdnerii kamloops,* taken from different environments or maintained under different experimental conditions and finds that only egg size is genetically controlled while egg number varies with environmental and physiological conditions. The possible significance of corpora lutea formation in restricting egg number is discussed below.

2. Sexual Cycles

A marked seasonal periodicity in egg production is characteristic of most fish. This seasonal cycle in activity of the gonad may commence before sexual maturity. Hickling (1935) found seasonal variation in the nuclear changes and egg diameter of immature hake. These cycles correspond closely in their season of incidence to the ovarian cycle of the mature and adolescent fish.

Seasonal changes in the reproductive activity of female sticklebacks, *Gasterosteus,* are shown diagrammatically in Fig. 2. In this particular case spawning occurs during a relatively short period between May and July. Following spawning, a wave of oocyte formation results in the differentiation of a large number of yolkless oocytes during the latter part of July, August, and September. Yolk formation commences in October and continues until just prior to spring spawning in May. The final maturation division occurs at the time of fertilization.

The length of the breeding cycle is extremely variable. Ovaries of the British hake are found in the ripe condition during every month of the

year except December. Haddock, on the other hand, spawn during a period of about 3 months (Hickling and Rutenberg, 1936). Under controlled laboratory conditions different species of viviparous cyprinodonts produce broods regularly for long periods at intervals of from 5 to 45 days (Turner, 1937). *Oryzias latipes* under such conditions spawns a few eggs daily. At the other extreme are fish such as *Squalus acanthias* which produces young biennially or teleosts such as *Anguilla* or the genus *Oncorhynchus* which spawn only once and die.

Both light and temperature have been shown to be important in controlling the reproductive rhythm of seasonal spawners (Fig. 3). In

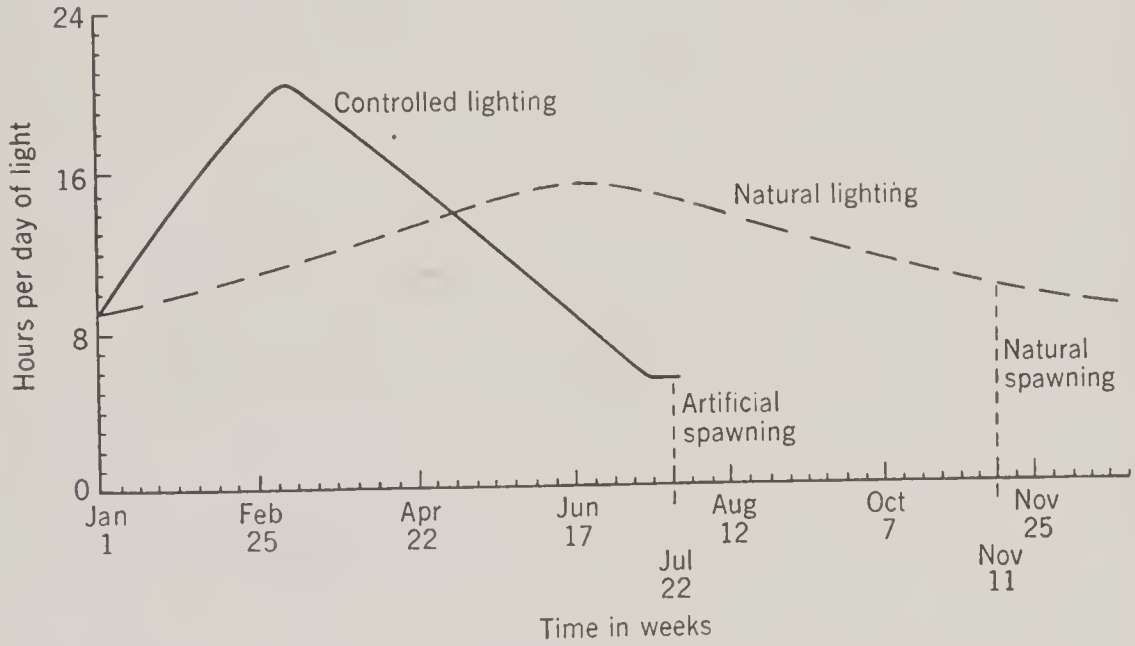

FIG. 3. Comparison of spawning time of brook trout accelerated by artificially controlled light with normal spawning time under natural light (Hazard and Eddy, 1951). (By permission of the American Fisheries Society.)

Gasterosteus appropriate manipulation of either light or temperature will affect the rate of gonad development but the response is much greater when these two factors reinforce each other (Chap. VI).

Sexual maturation may occur independently of changing light and temperature. Bullough (1940) found an internal rhythm in reproduction of *Phoxinus* which is reinforced and rendered more precise by seasonal changes but appears even under constant laboratory conditions. Many of the familiar aquarium fishes which reproduce so regularly in the laboratory show a seasonal reproduction in nature. There is thus a reproductive rhythmicity which is normally triggered by the environment but cannot be entirely suppressed by constant conditions. Virgin female *Lebistes* show an ill-defined cyclical production of eggs (Rosenthal,

1952), indicating that ovulation and gestation are not necessary to these rhythmic changes. A rhythm in production of pituitary gonadotropins may be expected (Chap. VI).

3. Fate of the Follicular Cells and Formation of Corpora Lutea [1]

The follicular cells which surround the developing ovum are arranged in several well-marked layers. In *Rhinobatus granulatus*, Samuel (1943)

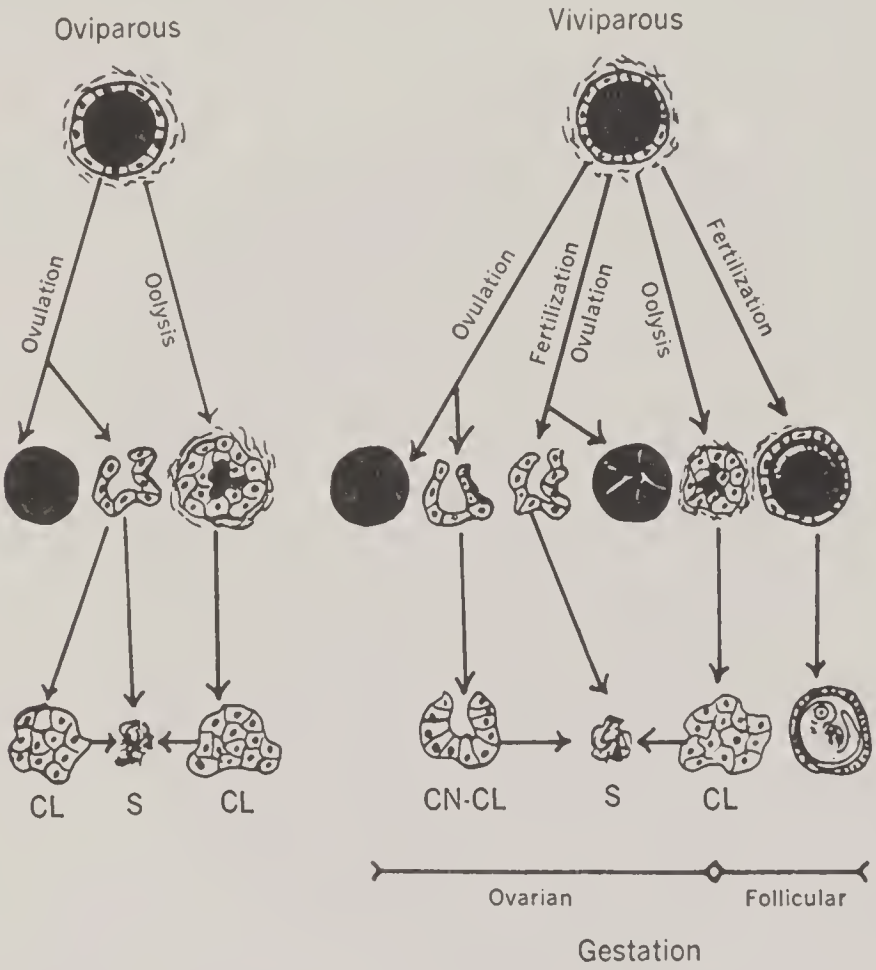

Fig. 4. The fate of the ovarian follicle in teleosts (Hoar, 1955). (By permission of the Cambridge University Press.)

describes an inner follicular epithelium (*granulosa*), an intermediate *membrana propria*, and an outer *theca* consisting of a compact *interna* and a loosely organized *externa*. A similar organization of the follicle has been described in the cyclostome *Myxine* (Lyngnes, 1936) and the teleost *Rhodeus* (Bretschneider and Duyvené de Wit, 1947). In some species, only a thin capsule-like fibrous layer can be distinguished (Cooper, 1952). Brock (1878), however, in his comprehensive com-

[1] It has been a privilege to discuss these problems with Dr. F. L. Hisaw.

parative work concluded that the teleost follicle always possesses a definite granulosa. It is probable that an inner granulosa and an outer theca are potentially present whether or not they are clearly marked.

The follicular cells have an interesting and varied history (Fig. 4). In all cases where a detailed examination has been made, some of the follicles fail to produce mature ova and the cells either become atretic or hypertrophy and develop into an enlarged glandular mass of cells which has been termed a preovulation corpus luteum (Fig. 5). This

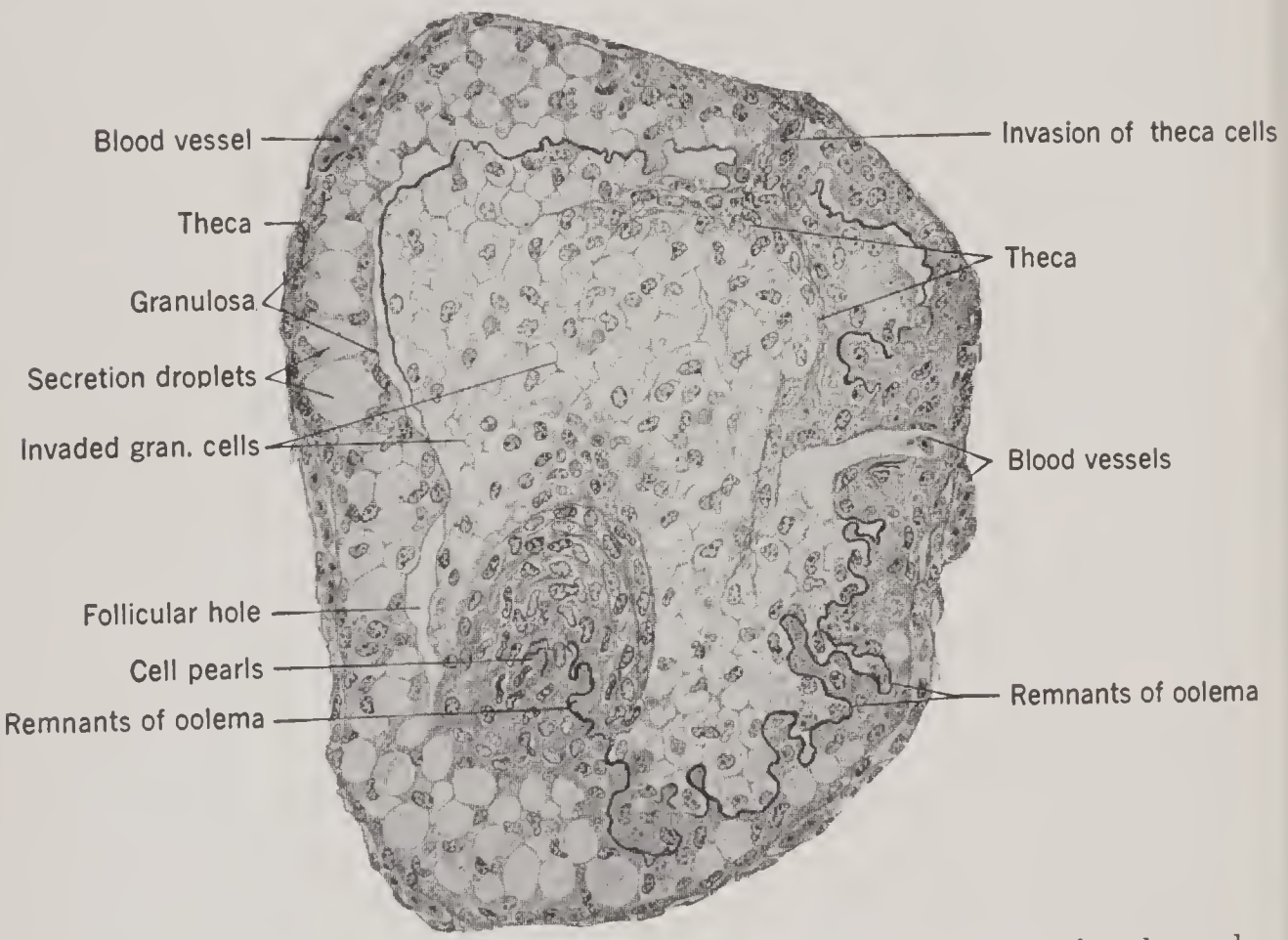

FIG. 5. Corpus luteum of *Rhodeus*, ready to secrete. From Bretschneider and Duyvené de Wit (1947). (By courtesy of Professor G. J. van Oordt, Utrecht, with the permission of the Elsevier Publishing Company.)

development has been carefully studied in cyclostomes (Lyngnes, 1936) and teleosts (Wallace, 1903; Bretschneider and Duyvené de Wit, 1947; Stolk, 1951). Bretschneider and Duyvené de Wit (1947) have made an exhaustive study of the histology and endocrinology of these bodies in the bitterling, *Rhodeus*, and described four distinct stages in their formation and eventual regression.

Several writers have noted the degeneration and atresia of developing

eggs and have speculated as to the significance and cause of this atresia. Others have emphasized the hypertrophy of the follicular eells, the apparently healthy condition of the resulting structure and the possible endocrinological significance. There is not yet sufficient information to establish the physiology of the follicular layers and their derivatives. The following facts, however, based on comparative work among the vertebrates, seem pertinent.

In mammals, estrogen production is attributed to the theca while progesterone is formed by the luteinized granulosa (Hisaw, 1947). In those vertebrates whieh produce yolk-laden eggs, the granulosa is responsible for yolk deposition. Furthermore, these same granulosa eells may actively phagocytize, or in some way remove yolk in follicles whieh are undergoing resorption. Thus, in the vertebrates as a group, granulosa cells may deposit yolk, or remove yolk, or seerete progesterone. It is also true that in all vertebrates numerous follicles which begin an apparently normal development undergo atrophy and fail to produee mature ova. The production of ova is dependent upon pituitary gonadotropins. Follicular atresia follows hypophysectomy (Hisaw, 1947).

It seems probable that the theca interna of the fish ovary likewise produces estrogens. Hisaw (unpublished data) found extremely high estrogen values in yolk-laden follicles and in eggs of the smooth dogfish (*Mustelus canis*) while the remainder of the ovary, containing numerous resorbing ova, was relatively low in estrogenie aetivity. The granulosa of the fish ovary, on the other hand, may or may not funetion in hormone production. Its function might be solely one of depositing and removing yolk. Hormone production by the granulosa may have evolved in the tetrapods. Harrison (1948) suggests that eorpora lutea appeared before placentation and later acquired their connection with placentation.

Whether or not the preovulation corpus luteum produces hormones, it is clearly an active organ and one whose formation is interrelated with changes in pituitary function. Hypophysectomy in the dogfish initiates yolk resorption and the development of these structures in all yolk-laden follicles (Hisaw, unpublished data). The development of preovulatory corpora lutea or eorpora atretica noted in several teleosts treated with steroids (Bretschneider and Duyvené de Wit, 1947; Rasquin and Atz, 1952) may be caused by a physiological hypophysectomy due to an inhibition of gonadotropin production by the steroids (Chap. VI).

A better understanding of the physiology of preovulation corpora lutea and atretic follicles might shed some light on factors eontrolling variation in egg number. Demands for pituitary hormones vary. It is possible that extended periods of appetitive behavior prior to spawning, perhaps involving long migrations, may greatly increase the demands for

gonadal steroids, thyroid hormone, or adrenal cortical steroids (Chap. VI). In this way production of gonadotropins would be stimulated if the gonads are involved or depressed if demands for thyrotropin or adrenocorticotropin are excessive. Relative numbers of preovulatory corpora lutea or corpora atretica and ripe ova may thus vary with the activities of several different endocrine glands whether or not the preovulatory bodies are concerned with hormone production. A search for the adaptive significance of variable egg number may find its answer in relative demands for gonadal or other hormones.

In addition to the preovulatory corpora lutea comparable bodies may develop from the follicular cells of the ruptured follicle (postovulation corpus luteum). These bodies have also been described in the several major groups of fish-like vertebrates as well as in the higher vertebrates. Details of the histogenesis have been given by Samuel (1943) for *Rhinobatus granulatus*, by Hisaw and Albert (1947) for *Squalus acanthias*, and elsewhere. Their association with "viviparity" seems evident in the elasmobranchs. In fish such as *Myxine* (Lyngnes, 1936), however, or oviparous teleosts such as *Fundulus* (Matthews, 1938) such a relationship cannot exist.

The use of the term "corpus luteum" may be questioned. It is true that in many cases these bodies are not yellow—at least during their most active stage—and, further, in many fish the most prominent development occurs in association with an oolysis rather than the ovulation which is characteristic of higher vertebrates. These bodies, however, clearly develop from follicular epithelium and theca in fish as in higher vertebrates (Fig. 5) and are consequently referred to here as corpora lutea.

In addition to the formation of different types of corpora lutea, the follicle cells play a very different role in some viviparous teleosts. In species showing follicular gestation the wall of the follicle becomes extremely vascular and develops villi for the nourishment of the embryo developing within its walls (Fig. 4).

4. Ovulation and Movement of Ova Prior to Fertilization

The ovulation process has not often been studied in fishes. Robinson and Rugh (1943) describe their observations on *Oryzias latipes*. *Oryzias*, like many teleosts, has a hollow ovary. Ovulation, as observed in this fish, is not cataclysmic but requires about 30 minutes. The protrusion, rupture, and emergence of the egg from its follicle are similar to these processes in the frog. The gradual increase in size of the egg and its follicle forms a mound-like protrusion. At ovulation the enlarged follicle ruptures and the musculature of the theca interna contracts so that the egg rolls out through the rupture like a big ameba. In the case of

Oryzias, as in most teleosts, the egg is freed into the lumen of the ovary rather than the body cavity as in the frog (Rugh, 1935).

The forces causing rupture of follicle are unknown. Although smooth muscle fibers are present in the ovarian wall, Robinson and Rugh (1943) did not note any movement of the wall. The controlling process may be assumed to be the same in fishes as in other vertebrates. Local changes in follicle are apparently induced by a delicate balance of the gonadotropic hormones of the anterior pituitary (Hisaw, 1947). In many teleosts ova are discharged into the cavity of a hollow organ or into a lumen, continuous with the oviduct on one side of the ovary (Brock, 1878). In cyclostomes, elasmobranchs, and teleosts which lack oviducts, the follicles rupture into the body cavity.

Robinson and Rugh (1943) believe that eggs are forced from the cavity of the ovary into the oviduct by the muscular movements of the ovary itself. The muscular oviduct and the muscles of the adjacent body wall may assist in a rapid movement of eggs through the short oviduct.

The ciliation of the abdomen has been carefully described in the dogfish and the movement of ova into the ovarian funnel observed under experimental conditions (Metten, 1941). In this case as in the case of the frog (Rugh, 1935) ciliation alone is sufficient to account for the movement of ova into the oviduct. In female salmon and trout, which lack an oviduct, ciliation of the abdominal cavity (developed under influence of gonadal steroids) has been described (Ashby, 1952) and may act in movement of eggs or circulation of coelomic fluids. However, contraction of abdominal muscles must provide the main force for the extrusion of the eggs from the body.

5. Nidamental Glands of Elasmobranchs

A specialized anterior portion of the oviduct secretes one or more nourishing and sometimes protective coverings as the ovum passes caudad. This area is referred to as the nidamental gland for its walls are specialized to secrete albumen, mucus, and horny shell material (Prasad, 1948). The typical oviparous form such as *Hydrolagus colliei* possesses an albumen-secreting zone anteriorly, a narrow intermediate mucus-secreting zone, and an extensive posterior shell-secreting zone. In typical ovoviviparous species such as *Rhinobatus granulatus*, mucus-secreting tubules are absent and the gland is smaller in size. The arrangement of the zones varies in different species. In *Raja rhina*, for example, the mucus-secreting zone is in a caudal position.

Metten (1941) finds spermatozoa in the shell-secreting area but not in the albumen and mucous areas. Metten believes that this area pro-

vides nourishment and spermatozoa may be stored here for some time before fertilizing eggs. Thus, the nidamental or oviducal glands of elasmobranchs also serve as seminal receptacles.

III. PHYSIOLOGY OF THE PRESPAWNING FISH

Sexual maturation involves the mobilization of relatively large quantities of nutritive material for the young. In addition, the development of the secondary sex characters may in some cases require profound reorganization of tissues or development of new structures. Curiously enough, these changes frequently occur during a fasting period (Fig. 6).

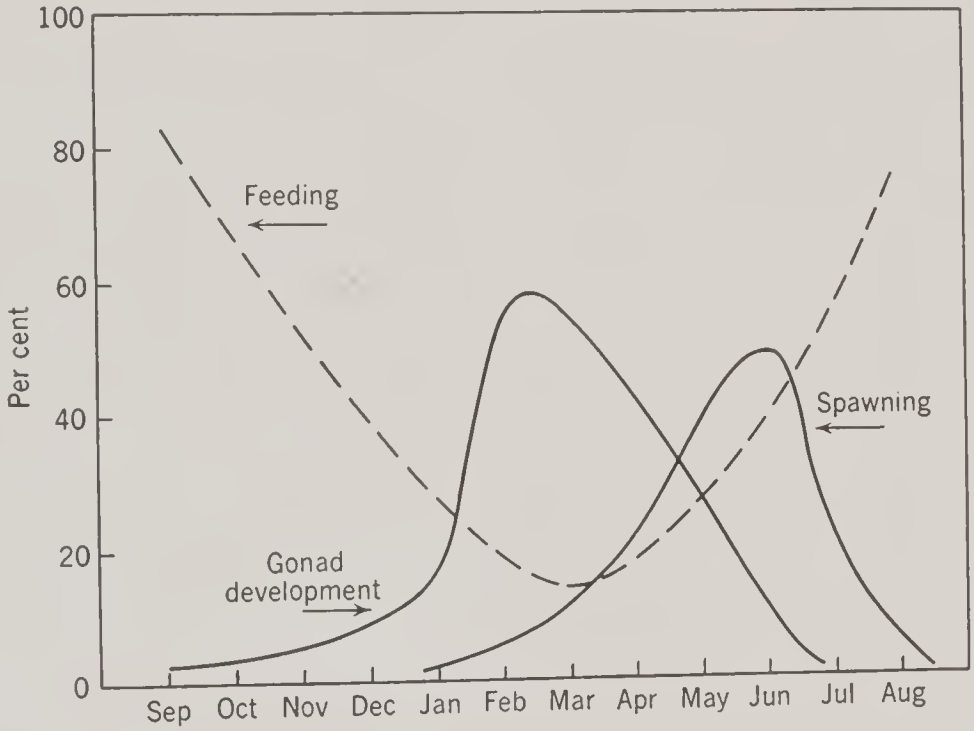

FIG. 6. Relation between feeding cycle (per cent sample with food in stomach) and reproductive cycle (gonad development, per cent fish with ripening gonads; spawning, per cent ripe fish) of haddock *Melanogrammus aeglifinus*. Data from Homans and Vladykov (1954).

The male pink salmon (*Oncorhynchus gorbuscha*), for example, during a period of from 30 to 45 days, at the end of a precise 2-year life cycle, develops a spectacular hump on his back which increases the depth of the body by 19%, the length of the head by 67%, and the width of the head between the nares by 34% (Davidson, 1935). These changes are largely due to development and growth of cartilage. Externally the female shows less remarkable changes but internally the eggs complete their development and are released into the body cavity. The fish take no food during this period. Less spectacular but comparable changes occur in most of the Salmonidae. In the Anguillidae a single spawning

is preceded by biochemical reorganization of many tissues. The ammocete of the brook lamprey, *Entosphenus wilderi,* does not metamorphose until the prespawning period and, like some salmonids, these many changes occur during a period of complete fasting which is followed by death (Okkelberg, 1921).

Greene (1926) reviewed the physiological literature of prespawning salmon. His bibliography includes references to the classical nineteenth century studies of Miescher and Paton on European Atlantic salmon. A translation of Miescher's important work is available to the English reader (Miescher-Ruesch, 1883). As Miescher pointed out long ago salmon may leave the ocean with a sufficient store of food to last them for 12 months and at the same time provide the stores which go into the eggs for the next generation. Such a long period of fasting is rather unusual even in the Salmonidae.

Accumulation and storage of fat prior to the maturation of the gonads is observed in many fish. In fish such as salmon and herring this storage takes place in the muscle, both within the fibers themselves and between the fibers, while in fishes such as the cod, storage occurs in the liver (Bailey, 1952). Feeding usually ceases prior to spawning and during this period of fasting the stored fat and, to a lesser extent, protein provide energy for prolonged migration and spawning activities. At the same time there is a transfer of protein and some fat to the gonads. Bailey (1952) discussed the changes in fat composition of several fish during the prespawning period. The fat content of the king salmon declines from about 15.5% to 2.2% while the oil content of Pacific herring declines from 19.8% to 4.1%. Different fatty acids may be selectively withdrawn or transferred. These data are summarized by Bailey (1952). Unlike salmon and herring, dogfish (*Squalus*) show little change in fat content during the reproductive period. However, the elasmobranch liver does change in weight during gestation. In species with an ovoviviparous type of gestation a reduction in liver size has been noted, while truly viviparous species show an increase (Ranzi, 1937).

Tables II and III show that the fats and proteins of the prespawning salmon are mobilized from muscle to gonads or used as sources of energy. The decrease of stored liver glycogen is also evident (Table IV). Greene (1926), however, did not find that the carbohydrate stores of either muscle or liver were sufficient to provide energy for the activities of migrating and spawning salmon. Fontaine and Hatey (1953) have re-examined the problem of carbohydrate metabolism in spawning salmon and the logical conclusion seems to be that gluconeogenesis from lipids and perhaps also amino acids is responsible for the carbohydrate required in the great muscular activity of migrating salmon. The loss

shown from the liver (Table IV) is almost equalled by an accumulation in the eggs (Fontaine and Hatey, 1953).

Fontaine and his group (Fontaine, 1954) have also studied changes in the iodine, copper, chloride, and calcium of maturing and spawning

TABLE II

CHANGES IN COMPOSITION OF SALMON (*Oncorhynchus tschawytscha*) DURING SEXUAL MATURITY

(Values in per cent of Fresh Wet Muscle Sample)

Composition	Tide Water	About 200 Miles from Sea	Spawning Grounds [a]
Water	63.2	63.4	79.7
Solids	36.8	36.6	20.3
Protein	17.0	17.0	13.7
Neutral fat	15.5	15.5	2.2
Phosphorized fat	0.9	0.8	0.4
Organic extractives	2.5	2.5	3.0
Ash	0.9	0.9	0.9
Muscle (per cent in standard fish)	71.6	—	32.8

[a] The fish from the spawning grounds had been in fresh water without eating for 4 to 5 months (Greene, 1926).

TABLE III

COMPARISON OF SALMON GONADS FROM ESTUARIES AND FROM UPPER WATERS OF SALMON RIVERS IN SCOTLAND [a]

Season	Muscle (% of standard ♀ fish)		Ovaries (% body weight)		Testes (% body weight)	
	Estuary	Upper River	Estuary	Upper River	Estuary	Upper River
May–June	62.7	63.9	1.2	2.9	0.15	0.44
July–Aug.	64.0	61.2	2.7	5.2	0.38	1.33
Sept.–Oct.	52.0	42.7	12.4	23.2	2.71	3.32

[a] Data of Paton and Dunlop as compiled by Greene (1926)

Atlantic salmon (Table IV). The accumulation of iodine in the form of thyroid hormone may play a vital part in the physiology of the salmon's return to fresh water. The anadromous lamprey, however, making a comparable change of osmotic media, shows no such mobilization of iodine. The matter has been more fully discussed elsewhere (Hoar, 1953, 1955).

Carotenoid pigments are in active movement during the development of the gonads and just prior to spawning (Goodwin, 1950). In male

Fundulus, xanthophyll is stored in the skin while in female *Fundulus* it is concentrated in the eggs. The bright colors of many fish at spawning time are due to the concentration of carotenoids and may be important in spawning behavior or have survival value as protective coloration. The reserve accumulated in the egg may be important in supplying chromatophore colors to the developing larvae. Recent work also indicates that the carotenoids of the egg play a vital role in fertilization by stimulating chemotaxis of the spermatozoa (Hartmann *et al.*, 1947; Brachet, 1950).

TABLE IV
CHEMISTRY OF TISSUES OF *Salmo salar* [a]

Composition of Tissues	Parr	Smolt	Adult	
			Maturing	Spawning
Iodine, serum, γ/100 ml.				
Total	10.1	33.5	124.2	21.5
Thyroxine	3.1	4.3	16.0	6.04
Protein	5.6	7.0	31.0	15.7
Copper, whole blood, γ/100 g.	42	53	64	44
Chloride, serum, g./l. NaCl	7.20	7.17	7.92 ♂	7.37 ♂
Freezing point Δ	—	—	0.75 ♀	0.66 ♀
Calcium, blood, mg./l.	144	155	174	138
Protein, serum, g./l.	—	—	69.5	33
Glycogen, liver, mg./g. fresh	18.22	1.50	27.14 ♂	24.86 ♂
	—	—	18.39 ♀	0.46 ♀
Chloride, muscle, g./kg. fresh	0.317	0.260	—	—
Lipid, percentage	3.9	1.2	—	—
Iodine value	148.5	166.0	—	—

[a] Compiled from various sources (Hoar, 1953).

IV. FERTILIZATION

In the majority of fishes fertilization occurs externally when eggs and spermatozoa are discharged in close proximity. The sexual products perish within minutes unless they unite. The complex and varied behavior patterns of fish which insure prompt union of sperm and egg are discussed in Vol. II, Chap. III.

In the evolution of viviparity and in the production of large eggs protected by horny shells an array of copulatory devices has appeared for the transfer of spermatozoa to the body of the female. Intermediate conditions are found where eggs are fertilized while still in contact with the female, or where fertilized sometimes externally and sometimes

internally (*Oryzias latipes*), or where fertilized in the body of the female and developed in the body (mouth) of the male (*Apogon imberbis*) (Needham, 1942; Garnaud, 1950).

A. External Fertilization

Under appropriate conditions fish spermatozoa may retain their fertilizing power for a considerable period. Salmon and trout spermatozoa, for example, are motionless in spermatic fluid and may be stored outside the body of the fish in this condition for many hours (Barrett, 1951). In water they become intensely active and survive for a brief period of only 1 to 2 minutes (Gray, 1920). On the other hand motility may be maintained for as long as 3 hours in 20% sea water or osmotically equivalent sucrose. They live many hours if diluted with peritoneal fluid (egg fluid) and stored at low temperatures (Ellis and Jones, 1939; Lindroth, 1947).

Spermatozoa of the marine teleost, *Gillichthys*, likewise showed longest motility in about 25% sea water or sucrose, but are active over a very wide range of salinities perhaps in association with the wide salinity tolerance of this species (Weisel, 1948). Although the osmotic content of the fluid giving greatest activity for both marine teleost (*Gillichthys*) and freshwater teleost (*Salmo*) are the same there is a real difference correlated with the environment since goby spermatozoa are inactive in fresh water and active in sea water while the reverse is true for the salmon.

Most of the interesting findings on the biochemistry of fertilization have been based on eggs and spermatozoa of invertebrates (Brachet, 1950). Hartmann *et al.* (1947) have, however, used trout material while Montalenti (1949) has investigated the gamones of *Lampetra fluviatilis*. From these studies it seems likely that future investigations will show that among fishes also several chemicals govern the union of eggs and spermatozoa. In several species of invertebrates and vertebrates it has been shown that both eggs and spermatozoa release two types of fertilizing chemicals (Brachet, 1950; Raven, 1954). In American literature the egg secretions are referred to as *fertilizin* and the sperm substances as *anti-fertilizin*. In European literature *gamone* is frequently used as a general term while the egg substances are specifically gynogamone I and II and the spermatic materials androgamone I and II. Gynogamone I accelerates the movement of spermatozoa and attracts them to the egg (chemotaxis) while gynogamone II agglutinates and holds them to the egg surface or destroys spermatozoa of other species by agglutination. Androgamone I depresses the activity of spermatozoa in the male genital

tracts so that they do not exhaust their energy and androgamone II dissolves egg membranes and counteracts the agglutinating action of gynogamone II.

1. Water-Hardening of Eggs [2]

Two phenomena occur when the soft teleost egg comes into contact with fresh water. The first is the lifting of a capsular membrane by the movement of water into the perivitelline space. The vitelline membrane evidently forms a barrier so that water does not penetrate the entire egg. It is presumed that both the capsular and vitelline membranes are present when the egg is extruded and only become noticeably separated after the entrance of water. The second process is the hardening or toughening of the capsular membrane. In *Salvelinus fontinalis,* water entrance is generally complete within an hour; hardening in about 24 hours. Eggs may take in water and yet fail to harden but in no case do they ever become hard unless some water has entered. The process of water entrance rather than the hardening of the capsular membrane has been shown to prevent subsequent fertilization of trout eggs. Fertilization does not affect either the amount of water gained or the degree of hardness developed.

The uptake of water and the "hardness" of the capsule may be varied experimentally. Eggs bathed in calcium chloride (0.0001 *M* and greater) become much harder than they do in distilled water. In distilled water they show about 60% hardness in comparison with eggs treated 24 hours with 0.001 *M* calcium chloride. Substances which remove calcium ions prevent hardening. Thus sodium citrate (0.01 *M*) or sodium oxalate (0.02 *M* and greater) keep trout eggs as soft as newly shed coelomic eggs. The degree of hardness may be varied by appropriate mixtures of calcium chloride and sodium citrate depending on the concentration of free calcium ions. Hardness developed in distilled water is probably due to the leaching of calcium ions from the egg itself. Both magnesium and manganese chloride harden trout eggs but are less effective than calcium. Manganese is more effective than magnesium.

The capsules of trout eggs, hardened with calcium chloride for 100 hours, do not become soft by subsequent treatment with sodium citrate. Further, if the eggs are kept soft with strong citrate solution for prolonged periods, the capsules tend to lose their ability to become hardened.

The force by which water is drawn into the perivitelline space of the trout egg is ascribed to osmotic pressure differences dependent upon the

[2] I am indebted to Althea Warren for this summary based on unpublished investigations reported in abstract by Manery *et al.* (1947) and Warren *et al.* (1947).

presence of colloidal substances in the perivitelline space. Water uptake can be controlled experimentally by immersing eggs in solutions of sodium chloride or other electrolytes. Although different electrolytes (sodium acetate, sodium thiocyanate, sodium iodide) exert specific effects they all show the same general action of decreasing the amount of water gained in proportion to their osmotic concentration.

B. Internal Fertilization

Of those animals which bring forth living young, it is customary to distinguish between ovoviviparous and viviparous forms. The former are usually nourished by the relatively abundant supply of yolk and draw only water and minerals from the parent while the latter receive all or almost all of their nourishment from the mother and have a specialized organ of attachment, the placenta. Such a variety of specializations are found among fishes that this distinction is often unsatisfactory. Embryos of some species receive all their nourishment from maternal secretions which may be produced by the wall of the uterus (Müllerian duct) or the lining of the ovary or the ovarian follicle. Various degrees of intimacy between maternal and fetal tissues are found in different forms. Structures comparable, physiologically, to the placenta have apparently evolved several times. Consequently no distinction between ovoviviparity and viviparity is made in this review. The word "viviparous" is used in the widest sense to describe the condition in which the female produces living young. The advantages of viviparity have been discussed by several workers (Needham, 1942; Matthews, 1955).

The majority of the Chondrichthyes are viviparous, although exceptions occur among both sharks and rays. All of the Holocephali are oviparous. Tortenese (1950) has discussed the evolution of viviparity among the cartilaginous fishes. Among the Osteichthyes, viviparity is confined to four families of the Cyprinodonts (Poeciliidae, Goodeidae, Jenynsiidae, and Anablepidae), to the sea perches (Embiotocidae), a few blennies (*Zoarces*), and the rockfishes (Scleroparei).

1. Chondrichthyes

In the Chondrichthyes internal fertilization is universal and transfer of spermatozoa from male to female effected by a special male copulatory organ, the clasper, developed from the basal element of the pelvic fin (basipterygium). Leigh-Sharpe (1920, 1926), in a series of articles has described various modifications in different species. In general the basipterygium is directed posteriorly as a stout skeletal rod supporting a portion of the pelvic fin. This rod is not a solid structure but is rolled to form a groove or tube whose edges overlap but do not fuse. The

anterior proximal opening of the tube (apopyle) is near the cloaca in sharks and dogfish but some distance removed in the skates. The posterior distal end of the organ is expanded into a fan-like rhipidion, an effective specialization for dispersing sperm in a radiating spray during copulation.

Numerous modifications of this basic structure are found in claspers of different elasmobranchs. The organ may be naked and smooth (*Galeorhinus, Raja*) or variously armed with denticles, spurs, or claws (*Squalus, Scyliorhinus*). Such spiny structures function in maintaining contact during pairing and may also serve to rupture membranes in virgin females. Vascular erectile tissue is found in the claspers of certain species. In many, this is a quite insignificant network of blood vessels in the rhipidion. In *Raja circularis* and *Galeorhinus* (Leigh-Sharpe, 1921), however, there is a minimum of cartilaginous tissue and much erectile tissue which may increase several times normal size during copulation.

A mechanism for pumping spermatozoa through the clasper groove or tube is a constant feature of this copulatory apparatus. This seems to have evolved from a muscular cavity in the proximal part of the clasper. The more or less unspecialized condition is found in some present day Holocephali and has evidently been superseded by the abdominal syphon (sharks), an extremely muscular sac filled with sea water or dilute lymph situated under the skin just anterior and lateral to the cloacal area. The Heterodontidae represent an intermediate condition between the Holocephali and the Euselachii in possessing both a cavity (nonfunctional) in the proximal part of the clasper and abdominal syphons. The skates and rays show a further specialization with a clasper gland developed inside the original syphon. This structure produces milky-white semi-viscous fluid which coagulates on contact with sea water or blood (Friedman, 1935). Leigh-Sharpe (1926) on basis of structure and origin considers the clasper glands of elasmobranchs to be homologous with Cowper's gland in mammals.

The spinal nerves supplying the clasper and gland can be readily dissected out and stimulated electrically to show their relation to the different reflexes involved in erection and copulation (Leigh-Sharpe, 1922; Friedman, 1935). Friedman (1935) found a nerve center for clasper erection within the spinal cord in the region of the 48th and 50th nerve centers of the skate. In erection the organ is brought forward by muscular contraction and its distal end or lips opened. Increased vascularity is evident and in forms possessing erectile tissue results in enlargement and turgidity of the organ. Contraction of the muscular syphon expels fluid into the tube or groove and carries the sperm,

discharged from the cloaca, into the female genital tracts. In the sharks only one clasper is evidently inserted during copulation but in at least some of the rays both are used simultaneously.

2. Teleosts

Among teleosts the urogenital papilla or anal fin may be enlarged and modified for the transfer of spermatozoa. Pelvic fins, tubercles, and other appendages are sometimes specialized and play a part in copulation. The mating of cyprinodont fishes has been carefully studied. The structures show a high degree of anatomical specialization. The anal fin rays of the male are modified to form an elongated copulatory organ, the gonopodium. This may contain a definite tube or groove to assist in the transfer of sperm. The fin rays of the gonopodium articulate with the vertebral column through a suspensorium of modified hemal elements and the movements of the structure are controlled by a powerful muscle which in *Gambusia,* for example, produces a perceptible bulging of the body wall just above the anus (Kuntz, 1913). Both males and females possess the genetic makeup for the development of a gonopodium and its associated structures but androgenic hormones are required for its expression (Turner, 1950). The comparative morphology and genetics have been examined (Turner, 1950; Gordon and Rosen, 1951; see also Vol. II, Chap. X. In *Lebistes,* insemination normally involves the use of both gonopodium and pelvic fins. Careful descriptions and photographs of copulation are available (Clark and Aronson, 1951; Clark and Kamrin, 1951).

Other groups of viviparous teleosts (such as the Scorpaenidae and Embiotocidae) lack the gonopodial specialization of the anal fin. Males may possess a thickening of the tissues at the base of this fin. Transfer of sperm must be effected through the genital papillae and cloacal contact. The details do not seem to have been described.

In many of the Cottidae the anal or genital papilla is greatly enlarged and may be directed forward as a stout penis in the male (*Icelinus, Clinocottus*). The Cottidae are oviparous. It seems likely that these papillae insure close proximity of eggs and spermatozoa at the time of spawning. In female *Orthonopias triacis* the protrusible oviduct is smeared with sperm in copulation and then retracted. Fertilization occurs as the eggs are extruded and pass, a few at a time, through this portion of the oviduct (Bolin, 1941). Erectile tissue has been noted in genital papillae of both males and females (Weisel, 1949). In *Apogon imberbis* the urogenital papilla of the female is enlarged and introduced into the male for the reception of sperm (Garnaud, 1950). In *Rhodeus* a much elongated urogenital papilla transfers eggs into the gill spaces of a fresh-

water mussel, *Anodonta*, where they are fertilized by sperm shed into the surrounding water and develop within the shell of the mussel. Thus a marked increase in size of urogenital papilla may occur as a secondary sex character in either males or females.

V. GESTATION

The evolution of viviparity has been discussed in an interesting review by Matthews (1955). This process has developed along quite different lines in the two classes of viviparous fish. The uterus, an expanded por-

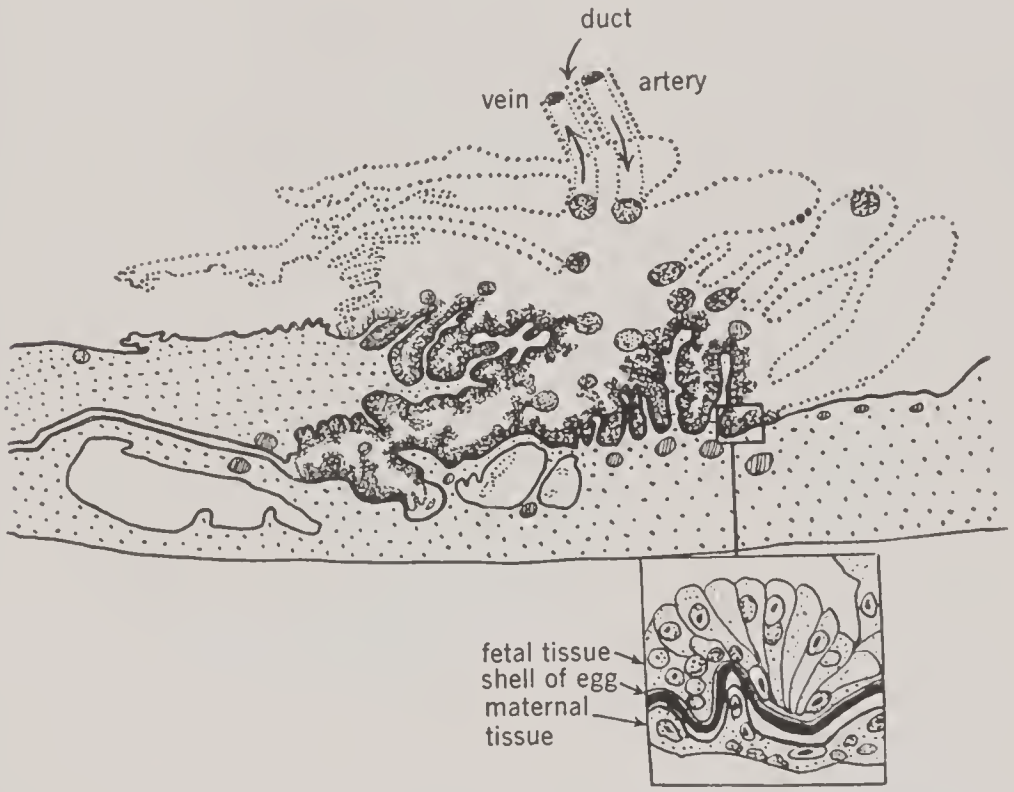

FIG. 7. Yolk sac placenta of *Mustelus*. Dotted outline, yolk sac; stippled area, maternal tissue; enlarged block, cellular detail of maternal–fetal union (from Ranzi, 1934, by permission of the Stazione Zoologica di Napoli).

tion of the oviduct (Müllerian duct), houses the developing elasmobranch while the viviparous teleost develops within the cavity of the ovary or within an ovarian follicle.

A. Chondrichthyes

Eggs of elasmobranchs, whether oviparous or viviparous, make their way as previously described from the abdominal cavity through the nidamental glands where the egg is fertilized before it arrives in the uterus. Gestation lasts many months and elaborate developments for nourishment and exchange of gases are required. Many of the interest-

ing details are described in a long series of articles by Ranzi (1932, 1934). A brief English summary of this important work and bibliography has been given by Needham (1942).

Ranzi (1934) has described the yolk sac placentae of *Mustelus* (Fig. 7), *Galeorhinus,* and *Carcharhinus.* In these fish, the endodermal epithelium of the external yolk sac is initially responsible for the digestion of yolk, while the elaborate circulatory network in the wall of the sac

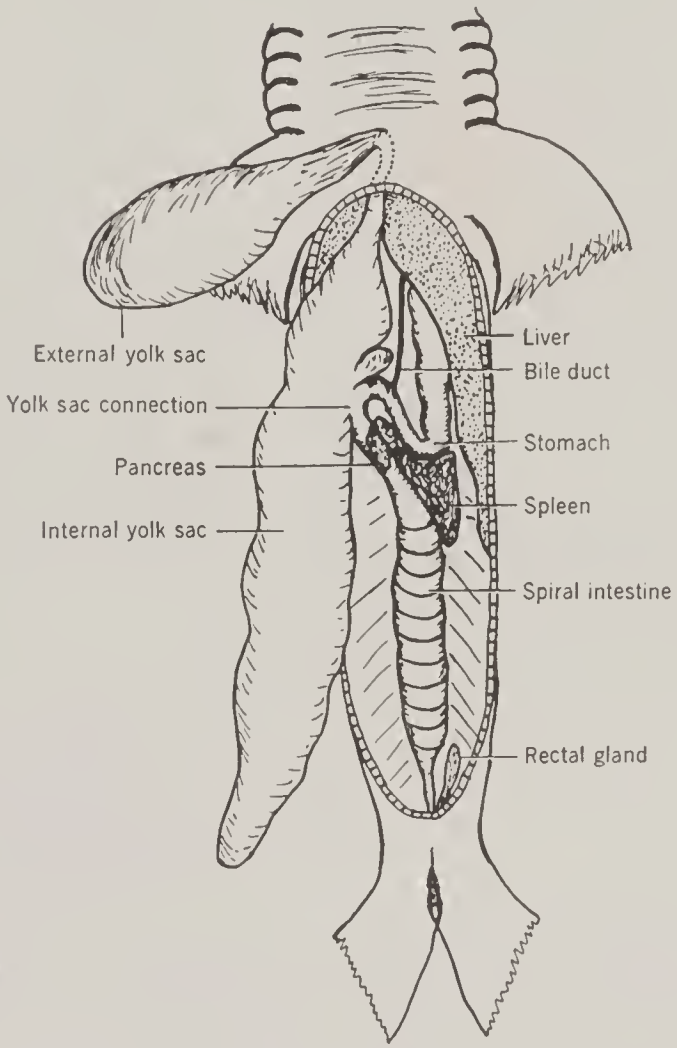

Fig. 8. Dissection of a 220-mm. embryo of *Squalus suckleyi.*

carries the digested material to the embryo and effects the exchange of gases. The uterine mucosa is smooth, loose, highly edematous, and the folds are wrapped around and closely applied to the embryo. Gestation lasts about 10 months in *Mustelus canis* (Te Winkel, 1950). During about two-thirds of this period a definite yolk sac placenta attaches the embryo to the uterus. A part of the yolk sac grows into the wall of the uterus so that the vascular channels of mother and fetus are in close contact. There is an elongated umbilical cord containing an artery, a vein, an extension of the coelom and intestine (Fig. 7).

The Squalidae possess an internal as well as an external yolk sac. The internal yolk sac is an expansion of the yolk stalk located within the body cavity of the embryo (Fig. 8). Yolk is moved by the ciliated lining of the external yolk sac and yolk stalk, through the internal yolk sac, into the intestine where it is digested and made ready for absorption by the intestinal villi and blood vessels. This function is initiated in *Squalus acanthias* when the embryo is 60 to 70 mm. in length. Digestive

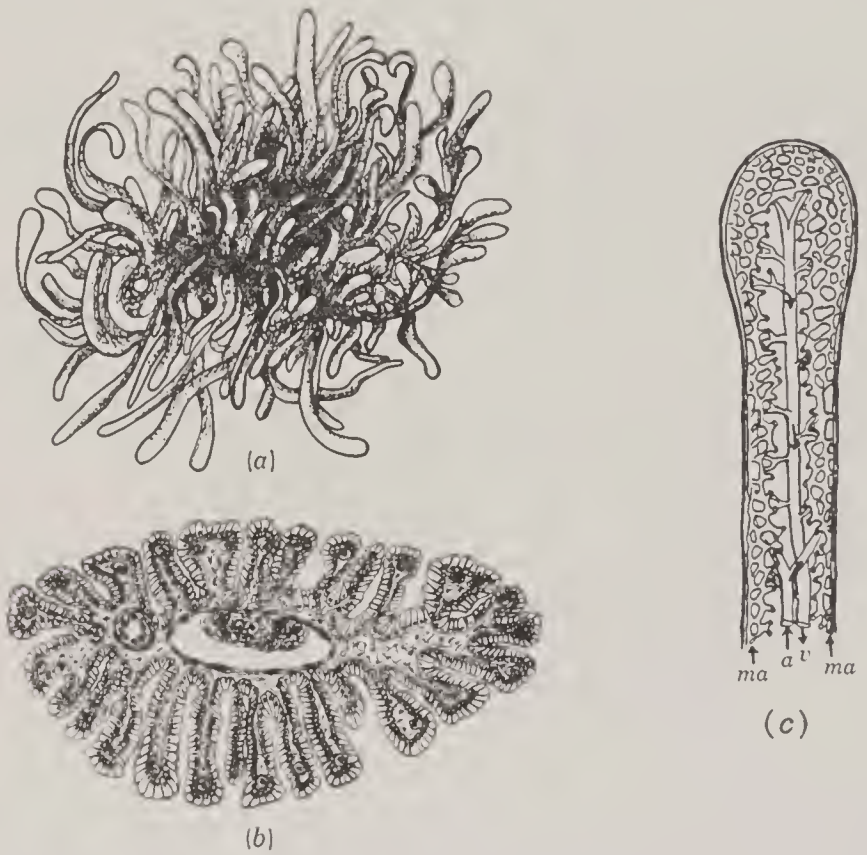

Fig. 9. Lining of the gestating uterus of *Trygon violacea*. a: portion of the inner surface of the uterus showing numerous villi (trophonemata); b: transverse section of villus (× 100) containing artery, vein, and capillaries; c: scheme of circulation in uterine villus; a, artery; ma, marginal artery; v, vein. Redrawn from Ranzi (1934) with permission of the Stazione Zoologica di Napoli.

juices of liver, pancreas, and intestine are thus responsible for the digestion of the yolk. The external yolk sac, which functions as a nutritive organ only during the early part of gestation, disappears before parturition. The young dogfish still possesses a small reserve of yolk in its internal yolk sac when it begins to take care of itself after birth (Te Winkel, 1943).

The Batoidea have evolved an entirely different nutritive mechanism. The yolk sac is very rudimentary and no placental development occurs. Instead the lining of the uterus forms a vast system of glands which

secrete an albuminous uterine milk aspirated into the oral region of the embryo through the spiracles. In *Trygon bleekeri,* Alcock (1892) found about 70,000 villi, termed *trophonemata,* lining the uterus. These structures varied in length from 0.5 to 1 inch and were in reality long compressed cones of blood vessels with glandular basal areas (Fig. 9). Nourishment by hydrotroph or uterine milk is evidently very efficient. A portion of the nutritive material may come from degenerating unfertilized eggs.

B. Teleosts

C. L. Turner's careful descriptions have provided a wealth of information on the maternal-embryonic arrangements in viviparous teleosts. The literature has been reviewed (Turner, 1947; Hoar, 1955). With one

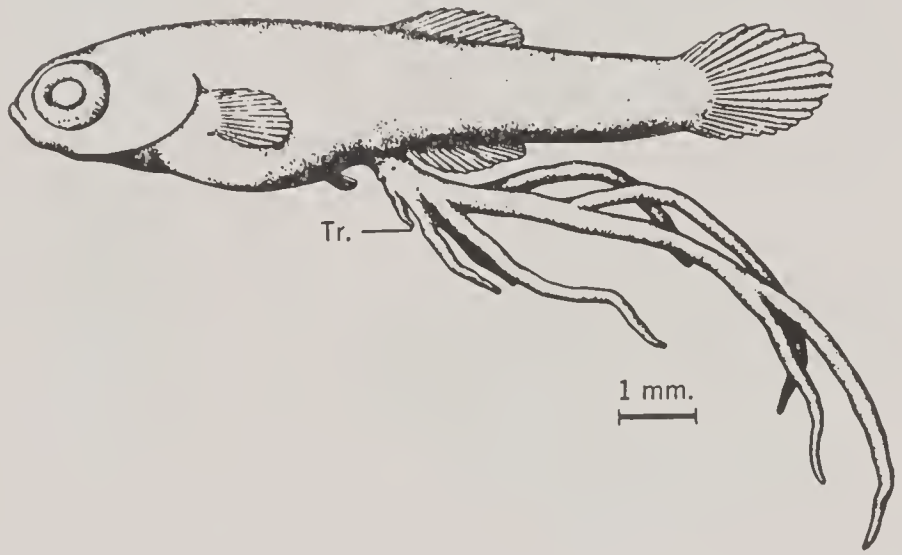

Fig. 10. Embryo of goodeid fish showing elongated trophotaeniae (Tr.) produced from anal lip. From original of Fig. 11 (Turner, 1940).

possible exception (*Zoarces*), fertilization takes place while ova are still within the ovarian follicle. All other species fall into two categories. The developing egg may ovulate during early cleavage and continue its development within the cavity of the ovary (ovarian gestation) or development may take place within the follicle until parturition (follicular gestation). Turner (1947) considers the former condition more primitive.

1. Ovarian Gestation

In ovarian gestation the epithelium of the ovary is highly convoluted, folded, hypertrophied, and glandular. These glandular, leaf-like extensions of the ovarian wall may grow into the gill cavities and mouth of the embryo (Jenynsiidae) to provide a means of transfer of material between embryo and adult. In the Goodeidae and *Parabrotula* special

finger-like or ribbon-like extensions from the anal or urogenital region of the embryo (trophotaeniae) become closely associated with the ovigerous folds (Fig. 10). In the Embiotocidae vascular extensions of the soft tissue between the rays of the vertical fins are responsible for the exchange of materials. As proof of the efficiency of this arrangement it may be mentioned that male *Cymatogaster* sometimes remain in the ovary until sexually mature (Turner, 1947).

Bretschneider and Duyvené de Wit (1947) ascribe secretory nutritive

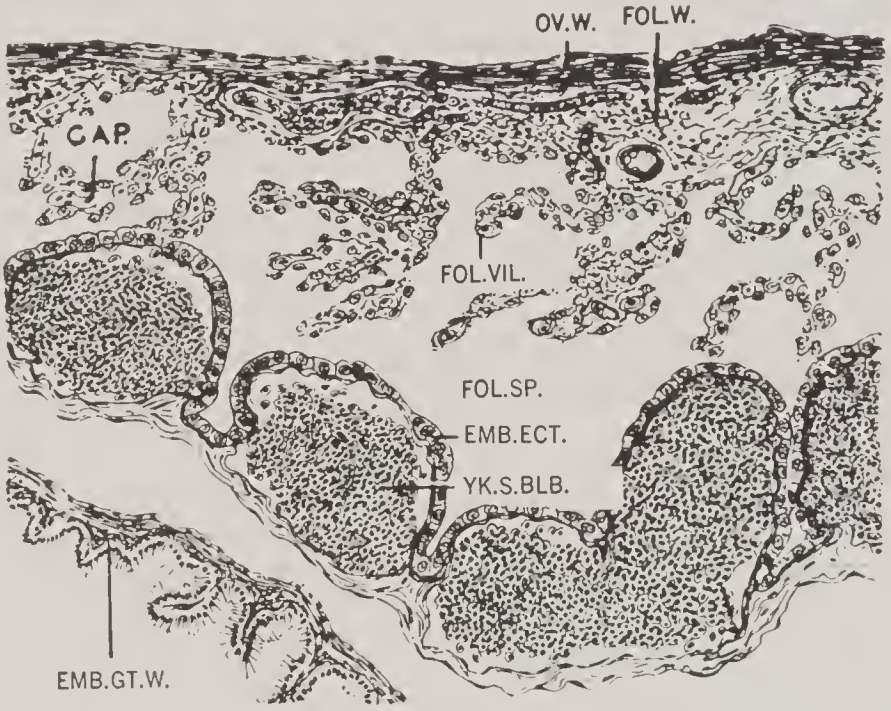

Fig. 11. Maternal fetal union in *Anableps*. The follicle wall (FOL.W.) is equipped with internal villi (FOL.VIL.), which contain capillaries (CAP.). A narrow space (FOL.SP.) separates the follicle wall from the external wall of the embryo (EMB.ECT.) with its rich vascular bulbs (YK.S.BLB.). The enlarged embryonic gut or intestine (EMB.GT.W.) lies in the body cavity. From original of Fig. 12 (Turner, 1938).

function to the follicular epithelium which persists as a *calyx nutricius simplex* in *Zoarces* after ovulation. This secretory material and mode of nutrition is comparable to that observed in elasmobranchs such as *Trygon*.

2. Follicular Gestation

In follicular gestation the maternal–embryonic connections vary with the quantity of yolk in the egg. If yolk is abundant, the follicular wall is fibrous, vascular, and intimately associated with an expanded pericardial sac of the embryo but there is relatively little modification of the ovary (Poeciliidae). If the yolk mass is small, both follicular wall and embryo show considerable modification to form a vascular, loose,

placental type of interconnection (Anablepidae). Villi formed in the wall of the follicle are in intimate contact with bead-like extensions formed in the pericardial or peritoneal sacs or the urinary bladder or intestine of the embryo (Fig. 11).

3. Superfetation

Fertilization within the ovarian follicle involves a penetration of sperm into the wall of the ovary (Dulzetto, 1937; Turner, 1947). In many species the epithelium of the ovarian cavity assumes a nurse-cell function where immobilized sperm, imbedded in the epithelium, remain alive for months. In *Heterandria formosa* live sperm have been found in the ovary 10 months after the female had a single contact with the male (Turner, 1947).

Superfetation seems to be a logical development of sperm storage in the ovary. The process has been described in several of the Poeciliidae (Table V). It reaches a climax in *Heterandria* where as many as nine broods may be developing simultaneously in the ovary and females produce young at intervals of about 5 days (Turner, 1937).

TABLE V

Sexual Cycle Showing Relation of Oocyte Development, Fertilization, Gestation, and Parturition in Several Poeciliidae [a]

		→ Growing Ovocytes → Fertilization → Retained Embryos → Birth
Gambusia	Brood A	——————————→
	Brood B	——→
Lebistes	Brood A	——————————→
Xiphophorus	Brood B	——————→
Platypoecilus	Brood C	——→
Quintana atrizona	Brood A	——————————→
	Brood B	——————→
	Brood C	——————→
Poecilistes pleurospilus	Brood A	——————————→
		(superfetation)
	Brood B	——————→
Poeciliopsis infans	Brood C	——————→
Heterandria formosa	Brood A	——————————→
	Brood B	——————————→
		(superfetation)
	Brood C	——————————→
	Brood D	——————————→
	Brood E	——————————→
	Brood F	——————————→
	Brood G	——————————→

[a] From Turner (1937).

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Chapter VIII

EARLY DEVELOPMENT AND HATCHING

Sydney Smith

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I. INTRODUCTION

Under the title of "Early Development and Hatching" this chapter will be concerned with the more recent studies of metabolism of the developing fish egg to the stage of development at which it is no longer dependent upon the yolk for nourishment or upon accessory food supplies derived from the maternal ovarian sac, as in the case of some "live bearing" teleosts, or upon oviduct wall secretion or placental transfer, as in the case of the elasmobranch fishes. It should be noted, however, that hatching in the case of numerous teleosts occurs some considerable period before the stage of dependence upon the yolk reserves has ended. Some consideration will also be given to the experimental study of fish embryology, that is "Entwicklungsphysiologie," in view of the widespread occurrence of anomalies of development in fish hatcheries. For accounts of the development and maturation of reproductory elements, breeding biology, influence of hormones upon the breeding cycle, and upon later development and growth, Chaps. VI and VII in this volume and III: Part 3 in Vol. II should be consulted.

A study of the literature shows that our knowledge of the physiology of early development is limited to a very small proportion of known fish, even in the case of those forms cultured in hatcheries and aquaria and of which material is accordingly available for controlled experiment. A small number of fish of commercial importance which lay pelagic eggs, for example cod and whiting, have received brief attention but most of the work is confined to the Salmonidae, *Fundulus*, and some few species of tropical fish easily reared in aquaria. The correlation and assessment of the results reported is rendered difficult because only in a few cases has it been possible to control conditions of development and experiment. For critical work it is essential that the population of eggs should be as uniform as possible both in size and in stage of development. Temperatures throughout development should be maintained at a uniform, preferably optimum, level, and all measurements of metabolism should likewise be made at this same temperature. It is clear that the resources of most fisheries research laboratories are such that these minimal requirements cannot be met even when the eggs are as abundant and as amenable to handling and sorting as are those of the Salmonidae. The reader is accordingly asked to recognize these inescapable difficulties at the beginning of this survey because they make a close and critical comparison of the findings of different workers impossible and often unreasonable.

The most generally accessible reference works are still the two major surveys of Needham, "Chemical Embryology" (1931) and "Biochemistry and Morphogenesis" (1942). Reference to Russian work is facilitated by the recent publication of Detlaf and Ginsburg (1954) on the embryonic development of sturgeons (in Russian). Review articles by Hayes (1949) and Oppenheimer (1947) deserve special mention as does the chapter contributed by Rudnick to the cooperative volume "Principles of Development" (1955); this deals with the experimental embryology of teleosts and birds.

II. THE FISH EGG

A. The Structure of the Egg and of its Membranes

Fish eggs from their origin as primordial germ cells in the ovary have two kinds of history, (1) in which the ovary alone is concerned in the formation of the egg and its protective membranes, and (2) those in which the egg, after ovulation, is covered by albumen layers and shell during its passage down the oviduct when it may or may not have already been fertilized (see also Chap. VII).

In the first case the oviduct may be reduced or even absent altogether, the eggs, after ovulation into the abdominal cavity, are expelled by a

short funnel opening by a pore, frequently closed by a membrane until the breeding season is imminent, or else into an ovarian sac with a separate duct and pore likewise guarded by a membrane. In some primitive fish, e.g. the lamprey, *Petromyzon*, the eggs are expelled through a urinogenital papilla. The arrangement is sometimes modified, internal fertilization taking place, and the egg is reared either in its ovarian follicle or in an ovarian capsule in which a secretion of considerable food materials to the egg may occur and finally a young fish is expelled to the outside world. In some cases structures analogous to a placenta and true placentae have evolved.

In the second case the egg is covered with layers of albumen in the oviduct, and later by a thick shell, secreted by a modified part of the oviduct wall (Prasad, 1951). The egg is usually fertilized internally shortly after ovulation. Often there then arise devices for the tubal incubation of the eggs, which accordingly may be reduced in size. This does not necessarily occur in all cases. The supply of food may be either by placental communication, close relations being established between the blood circulation of the yolk sac and the maternal circulation in the oviduct wall, or, more usually, by some elaborate "Histiotrophe" secretion of cellular detritus from the oviduct wall (Tortonese, 1950). These devices entail the reduction in thickness of the shell and modification in the amount of the yolk store. In cases where the eggs are laid, a considerable range of structure is found correlated with the conditions of incubation. Salmonid eggs, squeezed out and externally fertilized in a gravelly hurly-burly in the head waters of freshwater streams have a relatively thick and tough protective chorion, secreted within the ovary. Other teleosts, breeding in the sea, lay eggs which may be small and planktonic (pelagic); these are usually laid by bottom-living fish in large numbers, up to several million in the cod family (Gadidae). Fish of planktonic adult habit usually lay relatively smaller numbers of eggs which sink to the sea bottom to develop (demersal). Other teleosts with nest-building habits, mouth incubation, pouch incubation, and other highly developed protective mechanisms, lay much smaller numbers of eggs of relatively large size.

The physical and chemical description of the varieties of fish eggs will be considered in more detail in the next two sections. A brief outline of the changes undergone during development is given here because understanding of the stages of development is essential for what follows. After fertilization there is a more or less prolonged phase of cleavage. This is complete only in certain primitive fish of which the sturgeon, *Acipenser*, alone has been studied experimentally. In all other fish the cleavage is incomplete, there being a physically separate and distinct yolk. The boundary membrane of this yolk, and the devices to transport

the yolk into the embryo and convert it into organized tissue, find expression in much of physical and chemical interest. While cleavage proceeds in both elasmobranchs and teleosts the surface of the yolk is a noncellular membrane, the embryo consisting of a relatively small mass of dividing cells. Then follows gastrulation with a spreading of embryo tissue over the yolk, replacing the noncellular membrane by organized embryonic tissues. At the same time a localized infolding and accumulation brings about the establishment of an embryonic axis, and the young fish begins to take shape. Whether the embryonic axis is established and organized before or after completion of overgrowth depends upon the relative size of embryo and yolk. Then follows a period of steady growth, interrupted by hatching from the shell, during which the young embryo derives most of its food from the yolk sac. When absorption of this is nearing completion, the young fish feeds actively. The free-living yolk sac stage is often referred to as the "alevin."

B. Physical Properties

The passage from ovarian environment to coelomic and thence into the external medium, whether fresh or salt, subjects the teleost egg to a number of relatively violent stimuli, which result in profound changes in the physical properties of the surface membranes of the egg and of the protective shell. To a large extent independent of fertilization though simultaneous under natural conditions, these changes have been intensively studied within recent years, and will be reviewed in this section. No two teleost eggs seem to be exactly alike in physico-chemical behavior so the salmonid egg condition will be described first as the standard for comparison and then will follow an account of other teleost materials. The earlier salmonid egg studies by Hayes and co-workers are mostly reviewed in Hayes (1949) which surveys all work to that date on this material.

In the salmonid fish the eggs accumulate in the abdominal cavity complete with a soft shell secreted by the ovarian tissues. The shell has a small micropyle immediately under which is found a slight accumulation of protoplasm surrounding the egg nucleus. The full development of egg polarity has to await the stimulus of contact with fresh water. The containing membrane of the egg closely invests the surface membrane at this stage, and there is a considerable quantity of mucoprotein contained in cortical alveoli immediately beneath the egg membrane. These alveoli are not so numerous in the vicinity of the micropyle. Conditions in the coelomic environment are such that the eggs may be stored for a long time in a resting condition. It is probable that the level of respiration is exceedingly low at this time. Once the egg is shed through the abdominal pores into the hypotonic fresh water medium,

the micropyle remains open permitting sperm entry only for a short time. In the pike, *Esox*, the micropyle closes after ½ to 1 minute and this time is to some extent dependent upon temperature (Lindroth, 1947). The salmon egg loses 10% of sodium, 8% of calcium and chloride, and 16% of inorganic phosphate (Hayes *et al.*, 1946), the cortical alveoli discharge their mucoprotein contents [polysaccharide esters with sulfuric acid and amino sugars (Kusa, 1954)] into the space between the egg membrane and the chorion, and water is drawn in by the swelling of this material. This swelling takes 1 hour in *Salmo salar*, half an hour at 10–12° C. in *Salmo irideus* (Devilliers *et al.*, 1953) and 30 to 90 minutes in *Coregonus* sp. (Zotin, 1954). The initially soft material of the chorion is stretched by the swelling and becomes hard and rigid—the process taking some 30 hours to complete in the salmon (Hayes). The result of all this activity is the establishment of a considerable hydrostatic pressure in the egg contents. At the same time a streaming of cytoplasm towards the zygote nuclei is intensifying the incipient polarity of the egg, bringing about a bipolar differentiation which takes some 6 hours to complete. Finally cleavage begins. Lipid droplets accumulate underneath this polar cap of cytoplasm, and lipid bodies invade the surface membrane of the yolk (Mathur, 1950). The egg is relatively impermeable to salts from this time until gastrular overgrowth of the embryo tissues has replaced this membrane by the histologically differentiated, highly vascular tissues of the yolk sac. The toughened chorion permits the passage of ammonia, hydrogen, and hydroxyl ions, some potassium leaks out slowly, and a small intake of calcium and sodium occurs. Further water intake is prevented by the limiting size of the toughened chorion. The growing embryo is rather short of water in the period shortly before hatching. Hayes and Armstrong (1942) find the water content of the yolk in *Salmo salar* falls from 63.5% to 55.5% between laying and hatching. The chorion limits water entry for, if eggs are shed into distilled water, the perivitelline fluid volume is greater and the egg swells to a larger volume at which the chorion hardens (Devilliers *et al.*, 1953).

The chorion of the trout egg (Becker, 1928) is 33–37 μ thick, and is penetrated by numerous canals 1.37 μ in diameter running radially through it. The surface of the shell looks like a sieve, the substance of it is weakly doubly refracting. Colloidal materials and larger molecules are nevertheless retained by the chorion until hatching. Hayes (1942) and Smith (1947) independently observed a build up of amino-nitrogen compounds in the perivitelline fluid; these were released at hatching. Krogh and Ussing (1937N)[1] observed no penetration by deuterium oxide

[1] References in the text followed by "N" [e.g. Krogh and Ussing (1937N)] are not listed at the end of this chapter but may be found in the bibliography of Needham (1942).

into the trout egg until such time as the gastrular overgrowth was complete. While the passage of water was possible because D_2O entered the egg, there was no intake of water into the egg as a whole, wet weight being constant almost to hatching which does not occur until some 23 days after completion of gastrulation. Shanklin (1954) has shown in *Fundulus* embryos of post-gastrular stages, that their ability to survive and develop in salt concentrations varying from distilled water to double strength sea water, is dependent upon the integrity of their glycolytic mechanisms, which supply the energy necessary to maintain the dynamic equilibrium of the system. By use of marked ions, linear transport in both directions through the embryonic membranes was established. The gradient of ionic concentration across the egg membrane was changed by inhibitors of glycolysis such as fluoride and iodoacetate. The gradient for magnesium was inwardly directed, i.e., it is absorbed under normal conditions, while those for sodium, potassium, and calcium were directed outwardly; for each a plateau of exchangeability saturation could be established. In nature it seems clear that active transport mechanisms are operating in both directions, so that the embryo maintains itself at an osmotic pressure some 20–25% of the surrounding medium.

The conditions necessary for the development of bipolar differentiation in the rainbow trout egg have been studied by Devilliers *et al.* (1953). No increase in bipolar differentiation can be observed while the egg remains in coelomic fluid. In the 4–6 hours after transfer to water the cytoplasm concentrates in the embryonic disc to which yolk oil drops seem to be attracted. The formation of perivitelline fluid requires a contact of about 15 minutes with water; saturated air is not sufficient and nothing happens in contact with a nonaqueous liquid. Formation of the perivitelline fluid and bipolar differentiation of the germ proceed normally in distilled water and in solutions of mono- and bivalent cations of up to $M/20$ concentration. Inhibition of both processes occurs at $M/10$ concentration. Trivalent cations such as aluminum inhibit specifically the formation of the perivitelline space at $M/10$ and $M/20$ concentration though germinal segregation proceeds normally in such solutions. The subcortical granules of mucoprotein appear to be "tanned" *in situ;* their contents cannot escape. Ionic valency seems more important than either hydrogen ion concentrations or osmotic pressure. Surface active detergents, when not lethal, had no effect whatever on bipolar differentiation and the formation of perivitelline fluid. Kusa (1953, 1954) confirms most of these results for the salmonid *Oncorhynchus* and for the stickleback *Pungitius*. He finds a wave of discharge of cortical alveoli starting underneath the micropyle and taking 3 minutes to reach the vegetative pole. Unfertilized eggs when pricked with a needle show breakdown of alveoli at the site of puncture.

In the case of *Fundulus,* Kao and Chambers (1954) find the development of the internal hydrostatic pressure seems to require the prior discharge of the cortical alveoli. This can be initiated either by normal fertilization or by pricking the egg with a needle. The pressure takes about one hour to build up to its final value of 150 mm. Hg. A clear jelly-like colloid accumulates in the perivitelline space which finally occupies 40% by volume of the egg. Activation of the egg in hypotonic sucrose reduces the final volume of the perivitelline fluid and also the level of the hydrostatic pressure. In a solution of 0.2% phenol red the perivitelline space takes up the dye, which can be made to change color by adding HCl and NaOH alternately to the sea water outside. This shows that hydrogen and hydroxyl ions pass freely through the chorion.

Yamamoto (1953) working on the eggs of the flounder, *Limanda schrenki,* observes the formation of balls occluding the micropyle by a surface precipitation of the perivitelline fluid which leaks through. Unfertilized eggs showed them after 1 hour's exposure to balanced salt solutions of 1 *M* concentration or a mixture of sodium and calcium chloride of *M*/2. The size of the ball formed depended upon the composition of the solution. In 1*M* KCl a broad perivitelline space appeared though neither micropyle ball formation nor discharge of cortical alveoli could be observed.

In *Gasterosteus,* Thomopoulos (1953b) finds the eggs retained in a thick viscous ovarian fluid which is 1.5 times hypertonic to the salmon and perch body fluids. The genital pore of the female is closed by a membrane until egg laying. The egg shell is 35 μ thick and consists of one layer only. On laying, the formation of the perivitelline fluid is exceedingly rapid, taking only 3 minutes at 20° C. Bipolar differentiation is complete in 15 minutes and is accompanied every 60 seconds by great waves of contraction in the egg membrane. The egg is activated by pricking anywhere but, unlike other teleost material, the eggs are not activated merely by laying. In *Perca,* (Thomopoulos, 1953a), the egg is retained in the ovarian sac until laid, the genital pore being occluded by a fine impermeable membrane. The eggs do not suffer damage if the membrane is ruptured 5 days before the normal laying, but if this is done 20 days before laying the eggs are killed. The shell is in two layers, the outer, 50 μ thick, is thick and gelatinous, and the inner is 6 μ thick. Bipolar differentiation is begun before laying and is completed on touching water even though the egg is not fertilized. Unlike the trout, monovalent and bivalent cations have no effect whatever on the formation of the perivitelline fluid or on the concentration of cytoplasm within the germ in bipolar differentiation. Trivalent ions, as in the trout, inhibit formation of the perivitelline space without influencing bipolar differentiation. In *Ammodytes,* the shell, 19 μ thick, has an outer layer of 5 μ

pulled out into little tongues, and the egg, as in *Gasterosteus,* is not automatically activated on laying. Unfertilized eggs may develop a perivitelline space without any of the cortical alveoli being discharged. Eggs could be activated artificially by pricking or by surface active agents, such as saponin and commercial detergents, in a concentration of 1/10,000 in sea water. This is comparable to the activating effect of lipoid solvents on *Oryzias* eggs reported by Yamamoto (1951).

Movements of contraction in the early post-fertilization stage of teleost blastoderms have for a long time been studied by time lapse photography, Yamamotu (1931–1940N) Roosen-Runge (1938N), and Kuhl (1939). This study has been extended recently to trout eggs by Thomopoulos and Dragesco (1954) who cut windows in the trout egg chorion (*Salmo trutta* and *irideus*) and observe movements within the shell by time lapse photography. These are complex; waves of local contraction of the yolk sphere combine with general contractions to cause the germ at the morula stage to move in a slow circle of diameter equal to that of the germ taking about 3½ minutes at 10° C. Once epiboly has passed the equator this type of movement ceases, to show a slow rotation of the egg sphere about the axis, of a period of about 1 hour, which had apparently been going on all the time. Bright lights and high temperatures stop all these movements.

The specific gravity of the eggs of bottom-living marine teleosts is reduced during the late stages of maturation so that eggs and larvae float. The lipid droplet of the egg, being by no means of universal occurrence, has nothing to do with this flotation. The adjustment is brought about by the passage into the egg of a large volume of fluid secreted by the granular cells of the follicle which has a lower specific gravity than sea water. Fulton (1898) and Milroy (1898) have made a careful study of these changes, their figures for the plaice egg (pelagic) and herring egg (demersal) are as follows:

	Before Maturation		Ripe Egg	
	Volume in mm.^{3}	Specific Gravity	Volume in mm.^{3}	Specific Gravity
Plaice (pelagic)	0.928	1.070	3.48	1.025
Herring (demersal)	1.022	1.087–1.090	2.132	1.066

This fluid makes the yolk translucent as the yolk spheres coalesce. Fulton states "that inasmuch as the entrance of fluid in demersal eggs has no hydrostatic function, and is of no utility in connection with the external relationship of the ovum, the same change is probably of normal occur-

rence in the maturation of the ovum in other groups of vertebrates, and in some groups of invertebrates."

In these final phases of maturation the absolute amounts of nitrogen and phosphorus both increase, the percentage of total chloride and the absolute amount of total solids likewise increase. Phosphoprotein complexes develop and less diffusible bodies become more diffusible.

It is of interest to realize the importance of this volume change for the breeding habits of the fish. If the eggs were to mature simultaneously the female could not hold all the swollen eggs. Ovulation is therefore in small amounts and prolonged. The fish school so that males are continuously available to fertilize whatever eggs may be laid as they mature. In a dissected female cod, the volume of body (less the ovary) was 4.57 liters and the volume of ovaries was 1.7 liters which could produce 6.3 liters of mature swollen eggs.

In viviparous teleosts, the egg is fertilized within the ovarian follicle or else after ovulation into an ovarian sac. There is here no sharp change in environment. Whether perivitelline fluid is formed under these conditions has not been studied. It is certain that development within and transfer of nutriment through the chorion take place.

The eggs of the lamprey (*Lampetra reissneri*, formerly *planeri*) probably show a decrease in osmotic pressure when laid in fresh water. They certainly show inhibition of cleavage and shrink when exposed to *M*/7 NaCl solution, which is isosmotic to the blood and body fluids of the adult. (Yamamoto, 1952).

The physical properties of the perivitelline pellicle of the teleost egg are of the greatest importance in gastrulation. In *Fundulus* (Trinkaus, 1951), the surface gel layer of the egg is continuous and the cells of the epiblast of the germ are closely adherent to this. The gel layer is responsible for wound healing and can be isolated from the rest of the egg as an elastic film, the materials of which disperse in calcium-free sea water and in oxalate and citrate solutions. As gastrulation proceeds, the gel layer over the yolk passes into the endoplasm by dispersion in the vicinity of the periblast margin. The physical picture from this elegant study is not, however, the whole story, for Oppenheimer (1954) notes in the egg of *Gobius paganellus* half way through gastrular overgrowth a marked dumbbell shape difficult to reconcile with the scheme outlined above.

C. Chemical Composition

The chemical analysis of the fish egg has been pursued at various periods rather as an adjunct to other studies. The literature is accordingly somewhat scattered and the techniques used were not always such that comparison between the results of different workers is practicable. For

what they are worth the earlier analyses are well summarized and presented with encyclopedic documentation in Needham's "Chemical Embryology" (1931) and later relevant citations are given in the same author's "Biochemistry and Morphogenesis" (1942). The survey by Vinogradov (1953) gives much useful information on the eggs and embryos of marine fish. It is perhaps helpful to mention that most of this is to be found in Tables 289 to 311 of this monumental work.

The fish egg with its relatively bulky yolk is a most difficult subject for chemical analysis. For example the distribution of the total lipoid of the egg and the state of linkage of a substantial fraction to the yolk proteins is not to be solved by bulk analysis only (Smith, 1952). Information must be sought from the techniques of cytochemistry and more refined modern methods of electrophoresis and chromatography. Except for a brief note on the analysis of the yolk proteins of the Atlantic salmon by electrophoretic methods by Young and Phinney (1949), there are no studies known to the reviewer which use these methods; it is to be hoped more will be forthcoming.

As most of the chemical components important in relation to their metabolism during development will be discussed in Section IV, it is perhaps useful to give their occurrence in the egg of the salmonid, which is the material most used for carefully controlled work on the metabolism of the egg and embryo. Results of recent analysis of *Salmo irideus* are given herewith expressed in milligrams for a standard egg of 100 mg., which is a fair size for a rather large specimen from an English hatchery.

Salmo irideus: Amount for 100 mg. egg

Water	66.18	Hartmann *et al.* (1947)
Dry substance	33.82	Hartmann *et al.* (1947)
made up of		
Ash	1.29	Hartmann *et al.* (1947)
Fat (eiöl)	3.61	Hartmann *et al.* (1947)
Phosphatide fat	3.75	Hartmann *et al.* (1947)
Chorion	2.69	Hartmann *et al.* (1947)
Protein of egg contents	20.16	Hartmann *et al.* (1947)
Total protein	23.19	Hartmann *et al.* (1947)
Nitrogen-free extract	2.32	Hartmann *et al.* (1947)
Total carbohydrate	0.18–0.20	Smith (1952), Lafon (1947)
Carotenoids (fat soluble)		Steven (1950)
Lutein	1–2 μg.	
Astacene	7–12 μg.	

Further information on the chorion of the salmon egg is given by Young and Inman (1938N) who found 0.4% of ash and about 4.38% of volatile material. Arginine, histidine and lysine occurred in the respective ratios 4:1:3. Hayes and Hollett (1940N) found very little glucose

in the salmon egg, only 0.049 mg. for 100 mg. of egg. The rest of the carbohydrate is probably bound to protein.

Corti (1950) gives analyses of the essential amino acids of the rainbow trout egg. His material was reared at 15° C. and was analyzed after 28 days incubation. Hatching occurred on the 29th day and his eggs were therefore full of the accumulated end products of nitrogen metabolism which are, as Hayes and Smith have observed, unable to escape through the chorion. The percentages found of the fresh egg material were, arginine 1.77, histidine 1.07, leucine 3.20, methionine 0.91, phenylalanine 1.72, threonine 2.88, tyrosine 0.74, and valine 3.64. Tryptophan was destroyed during analysis.

D. Brood Size and Conditions of Incubation

It is particularly important for the physiologist in studying the fish egg to realize that the egg is only one phase of the totality of existence of his material; it is not an end in itself but merely the means of replacing the population losses from death and depredation. The over-all efficiency of reproducing mechanisms should accordingly be estimated as eggs surviving as breeding replacements in the population rather than as numbers of eggs in a clutch. The widespread occurrence of parental care, viviparity, and even placentation, reviewed by Wunder (1931N), Ranzi (1935N), Turner (1942), and Needham (1942) and in Chap. VII afford examples of true animal "economy" for it seems that the more efficient the protection of the young from predators the fewer the young produced at a time. The metabolic effort required to mobilize from body tissues materials sufficient for large broods of relatively bulky eggs, which are then left to fend for themselves, so characteristic of salmonid breeding, is usually fatal for the parents. The biological value of parents surviving to breed again and not wasting good food on laying eggs to be eaten by others is clear. Among teleost fish, salmonid breeding is primitive. Among the advanced teleosts the alternative to laying a few carefully tended eggs is to lay millions of very small floating eggs, from which pelagic larvae hatch and collect food from the plankton, being themselves important elements in the food chains of other fish. Freshwater plankton is not so suitable for this type of exploitation and freshwater fish either lay numerous large eggs, or numerous smaller eggs if spring or summer breeders, or fewer with associated nesting habits, or they adopt an extraordinary range of internal gestation mechanisms so that the young are released at a relatively advanced stage, capable of self-maintenance. In live bearers and in ovoviviparous and placentating elasmobranchs, mobilization of food reserves still has to take place but the demand is

spread over the whole period of gestation. Evidence for mobilization of food reserves is afforded by the "Rapport Hépatosomatique"—the ratio of liver weight to body weight (Olivereau and Leloup, 1950); a fall in this fraction usually arises from the effort in both sexes of producing ripe eggs or sperms and is effected by a reduction in the liver fats. In viviparous selachians the liver to body ratio falls during early gestation but this fall is arrested once a placenta is established; this appears, as in the mammalian decidua, to act itself as a store of food reserve material. In ovoviviparous selachians the fat reserves stored in the liver during sexual maturation are consumed during gestation and the weight of the liver falls. In the case of the California soupfin, *Galeorhinus zygopterus,* with 20–40 young at a time, Ripley and Bolomey (1946) report that females with no young have larger livers. Once the eggs are fertilized there is a short-lived increase in liver weight to approximately 20% of the body weight. The liver is engorged with oil which later in gestation is withdrawn so that the liver may be only 2% of the body weight, though figures of 3–6% are more usual. The vitamin A content of the liver hardly varies at all, being not used either in maternal or embryonic metabolism.

In livebearing fish, *Sebastes,* young are in the shell for a prolonged part of their intraovarial life (Lüling, 1951), and *Lebistes* young, dissected from their ovarian follicles, have been shown to be capable of rearing in non-nutrient Ringer solutions, though the addition of glucose was of assistance (Trinkaus and Drake, 1952). It is highly probable that *in vivo* these embryos require little if any nutritional contribution from the mother. This is not the case in *Cymatogaster aggregatus* where the egg is only 0.2 mm. in diameter and the young at birth are at least 34 mm. long. This egg is so small that the yolk mass is no larger than one of the blastomeres at the two-celled stage. The early work of Eigenmann (1894) on this form should be continued.

The correlation between brooding habit and egg size and number is shown in two species of *Apogon* (Garnaud, 1950). In both instances the male carries the eggs in the mouth and does not feed while doing so. In *A. imberbis,* with egg diameter 0.5 mm., there is a brood of 22,000 eggs, whereas the Australian species, *A. conspersus,* with egg diameter 4.5 mm., has a brood of 150 eggs only.

III. THE EFFECT OF ENVIRONMENTAL CONDITIONS UPON DEVELOPMENT

A. Temperature

Needham (1942, p. 331) remarks that "acceleration of embryonic development with rise of temperature is in itself a banal phenomenon, and its mathematical analysis leaves us only with further enigmas."

His further comment that . . . "only when temperature changes dislocate the developmental processes in an observable way can any progress be made" should be kept in mind when considering the effects of temperature upon the development of fish eggs.

At the beginning of development it is essential that the temperature should not be too low or too high. Makino and Osima (1943) report that refrigeration of the eggs of the carp (*Cyprinus carpio*) after they have been fertilized leads to the production of a diploid egg nucleus and consequent polyploidy of the zygote. The occurrence of polyploidy from such a cause in nature is unlikely as neither ovulation nor fertilization would occur under such conditions. Thomopoulos (1954) reports that in the egg of *Ammodytes* a temperature of 6° C. (in nature the temperature is 10–12° C.) slows down, by approximately 10 times, the processes of activation, bipolar differentiation, development of the perivitelline fluid, and the hardening of the chorion. Conversely temperatures of 20–24° C. block early cleavage stages in carp eggs at about 32 blastomeres (Lieder, 1953).

Details of the morphological structure of the fish when developed show changes in relation to the temperature at which the incubation takes place. The characters most affected are the so-called "meristic" ones, i.e. numbers of vertebrae, of finrays, and spines and the like, which all show independent variation. The most recent study is that of Lindsey (1954) on the developing eggs of *Macropodus opercularis;* the extensive earlier literature has been reviewed by Tåning (1952). Ljubitzky (1935N) studied the effect of temperature on the rates of early development of the eggs of *Salmo trutta* and *"fario";* the Q_{10} was low during cleavage, maximal shortly before gastrulation and showed violent alternations in level during the period of gastrular overgrowth. He concluded that periods of low growth rate were correlated with a high rate of embryonic differentiation. Temperature change clearly produces a wide variation in mortality as *Coregonus* eggs develop (Oliphan, 1954). Cod eggs (Bonnett, 1939) are much more resistant to temperature change once the blastopore has closed. A partial explanation of some of these findings might arise from the arrest by high temperatures of the normal movements of contraction and rotation of the germ within the shell; in the trout egg these are supposed to assist in respiration.

The careful study of salmon development at a wide range of temperatures by Hayes *et al.* (1953) shows that, within the limits of survival, extreme environmental temperatures can cause significant changes in the order of appearance of certain anatomical features. Hatching, for instance, is relatively precocious in relation to other characters at low temperatures and the development of eye pigment more precocious at

higher temperatures. Development in short can be regarded as the successive deployment of a chain of reactions linked in sequence. These chains branch and the effect of temperature is to encourage some of the branches more than others. This clearly lies at the base of the phenomena reviewed above, but this remark does not get us much further, in view of the extreme complexity of the developing embryo. The temperature effect on the efficiency of growth and the utilization of substrate materials will be considered in Section IV under general metabolism.

B. Light

The inhibitory effect of light upon development in the trout first remarked by Johansen and Krogh is well known. The varying response of the later stages of development has received a careful study by Stuart (1953) using *Salmo trutta*. For 72 hours after hatching the young fish lie on their sides and show no response to a light gradient. After this they move simultaneously away from light and when lit from above they burrow under the gravel assisted by a copious mucous secretion of the epithelium. Shortly before the yolk sac disappears completely they face the light and feed when offered food, especially when it is live or moving. Alevins responding negatively to light do not swim against the current, once they respond positively they orient against the current. Ultraviolet light (Hinrichs, 1925) is extremely lethal to the eggs of *Fundulus* shortly after fertilization and those which survive show a considerable frequency of anomalies such as cyclopia, twinning, and tail defects. Exposure later in development may delay hatching though the period of hatching is reduced. Haempel and Lechler (1931) find in salmonid fish an acceleration of hatching, however, but confirm the reduction of hatching period. Sensitivity varied with the type of egg. They estimated the intensity of ultraviolet of high summer midday under natural conditions would be fatal to salmonid eggs and larvae.

C. Other Factors

A brief note by Rugh (1954) reports the differential sensitivity of *Fundulus* eggs to graded doses of x-rays. Early cleaving stages were most sensitive, the egg during gastrulation required a high dosage to produce any effect. The most sensitive structures were the fore brain; Rugh was unable to confirm earlier reports that the tail was likewise affected.

The claim that high concentration of carbon dioxide led to high mortality in the eggs of the Pacific herring was found by Kelley (1946)

to be without experimental foundation. On the same species, McMynn and Hoar (1953) find that eggs and larvae tolerate a wide range of salinity change without any effect on the rate of development. Eggs would not survive in pond water until after gastrula overgrowth was complete. It was considered probable that the egg membrane was permeable to water and the change in tolerance brought about by gastrulation is quite consistent with evidence from other fish material reviewed under Section II, B.

IV. THE METABOLISM OF THE EGG AND ALEVIN

A. Nutrition in General

The concept that energy sources for embryonic development form an ordered sequence of carbohydrate, protein, and fat was developed by Needham (1931) in his "Chemical Embryology," Section 7.7; and further evidence in support of this view is briefly considered by him in Section 3.37 of his "Biochemistry and Morphogenesis" (1942). Further study of teleost fish material has tended to modify this concept and the earlier findings are discussed in Hayes' (1949) review. It is clear from the histochemical and cytological studies of Mathur (1950) that lipoproteins and phospholipids are absorbed by the rainbow trout egg within 1 day of fertilization and Yamamoto (1951) and Nakano (1953) have both discovered evidence of early use of fats in the egg of *Oryzias*. If the consumption of carbohydrate is deduced from a fall in general level of total carbohydrate in the egg, there is no conclusive evidence of significant combustion until gastrulation at the earliest (Daniel, 1947 in *Salmo salar*), or more probably during the establishment of the blood circulation (Smith, 1952 on rainbow trout). The most important food reserves for teleost eggs are proteins of the egg yolk combined with phospholipoids and probably metabolized in that combination; the conspicuous, carotenoid-pigmented glyceride fat droplets are not metabolized until a late phase of the yolk sac period, shortly before the yolk is entirely consumed and about the time when the larva is ready to take food. The sequence to be accepted in salmonid fish development would be lipoproteins direct into the germ followed by phospholipoids and then possibly some proteins and carbohydrate. Protein combustion is dominant for the hatching period and at the same time there is a marked fall in the general level of total carbohydrate. After hatching when the major growth phase is in train the substrate is phospholipoprotein of the aqueous phase of the yolk and not until this has largely disappeared does the combustion of the triglyceride fat of the yolk begin.

The amounts and proportions of food materials consumed during development can be estimated by analysis of total quantities in a selected population of eggs at the beginning and end of development. The beginning gives little trouble but the decision when the end of development is reached in the case of the salmonid alevin is a considerable problem. After the yolk sac has been entirely absorbed is rather too late because conditions of starvation are already apparent in the embryo. Judging when the external yolk sac is no longer visible is rather a matter of aesthetics of larval embonpoint. It is perhaps best to choose the point at which the rate of growth of the embryo is zero. This is about the 75th day of incubation at 10° C. for the rainbow trout. The food substances consumed by the rainbow trout can be seen from this summary (data from Smith, 1947, 1952).

Estimates from Analysis of the Egg and the Completed Embryo (in grams)	
Dry Material 100 eggs	3.00
Dry material 100 embryos, 75 days	1.818
Dry weight lost during 75 days development	1.182

Estimates from Metabolism Measured (in grams)	
Protein loss (Total N excreted multiplied by 6.25)	0.498
Fat loss calculated from total heat production less heat production from protein at 9000 cal. g.	0.555
Carbohydrate	0.030
Hatching loss	0.115
Total	1.198

The agreement between the two methods is quite reasonable. The percentage efficiency of development from these figures is

$$\frac{1.82 \times 100}{1.82 + 1.20} = 60.2\%$$

Lafon (1947) does not find quite such a high proportion of lipoid consumed. He was, however, only examining a limited number of stages, four in all, and his last measurement was made when the yolk sac was only half consumed. The main glyceride fat combustion was still to come. Hollett and Hayes (1946) find in the salmon egg a combustion of rather more protein in relation to fat. Their figures in milligrams per embryo of material burned were protein 10.8 and fat 7.2, the amount of these substances stored being, respectively, 12.6 protein and 2.2 fat. Thus, of the material in the egg, 45% of the protein was consumed and 77% of the fat. It is clear therefore that protein is a very significant source of material both for the formation of embryo and as fuel for development. Fat is relatively more important as a fuel.

Findings from fishes other than Salmonidae are few. Nowak (1941) in the carp egg finds a dry weight decrease in relation to the nitrogen content. Fats and carbohydrates must therefore be consumed in the period from fertilization to hatching. It seems also probable that the end products of nitrogen metabolism do not diffuse through the shell to any considerable degree. After hatching there is a further fall of dry weight in relation to the nitrogen content. In the alevin stage Nowak finds the yolk sac is used up entirely 5 days after hatching and it is only during the 3rd and 4th days that any fat is consumed. It seems therefore probable that the picture built up from studies in Salmonidae is generally valid for other teleosts.

B. Respiration and Heat Production

The respiration of the fish egg during maturation and fertilization has been measured by the Cartesian micro-diver respirometer using single oocytes, mature eggs, and newly fertilized or activated eggs of *Oryzias latipes* (Nakano, 1953). The growing oocytes show a respiration which increases in proportion to the volume of the oocyte. The ripe unfertilized egg has a much lower respiratory rate, which changes from the high level of the growing oocyte during the final stages of maturation. The respiration of the unfertilized egg stays constant when removed from the ovary and even in distilled water, though it is no longer capable of activation or fertilization. There is no sharp increase in respiratory rate on fertilization, but after staying roughly constant for two to three hours there is a rapid increase in the respiratory rate resembling the exponential rise found in sea urchin egg development.

Ishida (1951) has studied the effect of blocking oxidative phosphorylation in the same material by treating the fertilized eggs with 2,4-dinitrophenol. Development is inhibited but the rate of oxygen consumption of the cleaving eggs is greatly increased. There is no increase on treatment during the second day of development when the eggs show optic vesicles. The retardation of development and the rise in rate of oxygen consumption under treatment appear to be related. Phosphorylation appears to be a part of the system by which oxidative metabolism is geared to the synthetic activity of growth and differentiation. Upsetting the gearing leads to greater destructive catabolism, materials not used are burned.

A wide range of reagents has been applied by Hishida and Nakano (1954) to analyze further the intermediary metabolism in developing *Oryzias* eggs. They conclude that the glycolytic system, tricarboxylic acid cycle, and cytochrome system are all functional and development is accompanied by a corresponding increase in the amount of respiratory

enzymes. Their figure for the respiratory quotient at the beginning of development is about 0.75 and it rises during gastrulation to about 0.92 to fall in the later stages of development to about 0.7. This result suggests an early combustion of fat or protein or a combination of the two as main substrate, glycolysis only predominating during gastrulation or perhaps even later (Section IV, A). The capacity for anaerobic glycolysis rises steadily throughout development.

Early stages of fish eggs react differently to anaerobic conditions. Large eggs such as the trout resist relatively prolonged periods of oxygen lack, partly because they have a store of oxygen in solution in the perivitelline fluid, but also because the level of their respiration is low. Smaller eggs are not so resistant to the effects of anaerobiosis, though it has long been known that *Fundulus* eggs would continue to cleave for a while and then survive in a state of arrested development for four days under a complete vacuum. On readmitting air, normal development was resumed. Cyanide seems to have almost the same effect on *Fundulus* eggs; they develop without a functioning cytochrome system. Much smaller teleost eggs show block of mitosis by cyanide and they soon break up under treatment. In rainbow trout eggs, Devilliers and Rosenberg (1953) find pre-gastrular stages unaffected by anaerobiosis for 48 hours; after 96 hours they lag only 24 hours behind aerated controls. After 7 days they die as early gastrulae. Gastrulation and increased oxygen consumption appear related. The mid-gastrula does, however, grow for 24 hours and is blocked at the early neurula stage. Such arrested embryos can withstand up to 2 days anaerobiosis without irreversible damage. The degree of oxygenation in the natural spawning beds of the Chum salmon has been measured by Wickett (1954) who establishes a demand of up to 0.0003 mg. O_2 egg/hour which is not always satisfied under natural conditions. Eggs and larvae seem able to reduce their metabolic demands to survive periods of low oxygenation. The blastoderm movements described in Section II, B might also assist diffusion in the earlier stages.

The respiration of salmon eggs in the later phases of development before hatching has been studied recently by Hayes *et al.* (1951); working at 10° C. they measured the oxygen consumption using a dropping mercury electrode. Their readings extend from the 19th day up to the 50th day when the eggs began to hatch. The total oxygen consumed was estimated at 1400 $mm.^3$ per egg. The weight of embryonic tissues at any stage was estimated by dissection after formaldehyde fixation. At all stages the value of the oxygen consumption per gram of dry embryo tissue fluctuated at random between 131.5 and 156 $mm.^3$ O_2/hour. As the eggs developed the oxygen demand per egg increased, and

for the final two-thirds of the period to hatching, the oxygen figures could be described by the same type of equation as those for embryonic growth. Shampardina (1954) finds a peak in the O_2 consumption per gram of embryo at the end of the yolk sac phase in pike.

Priwolnev (1938) has found in *Salmo salar*, at 10° C., a partial correlation between oxygen consumption, measured by Warburg respirometers,

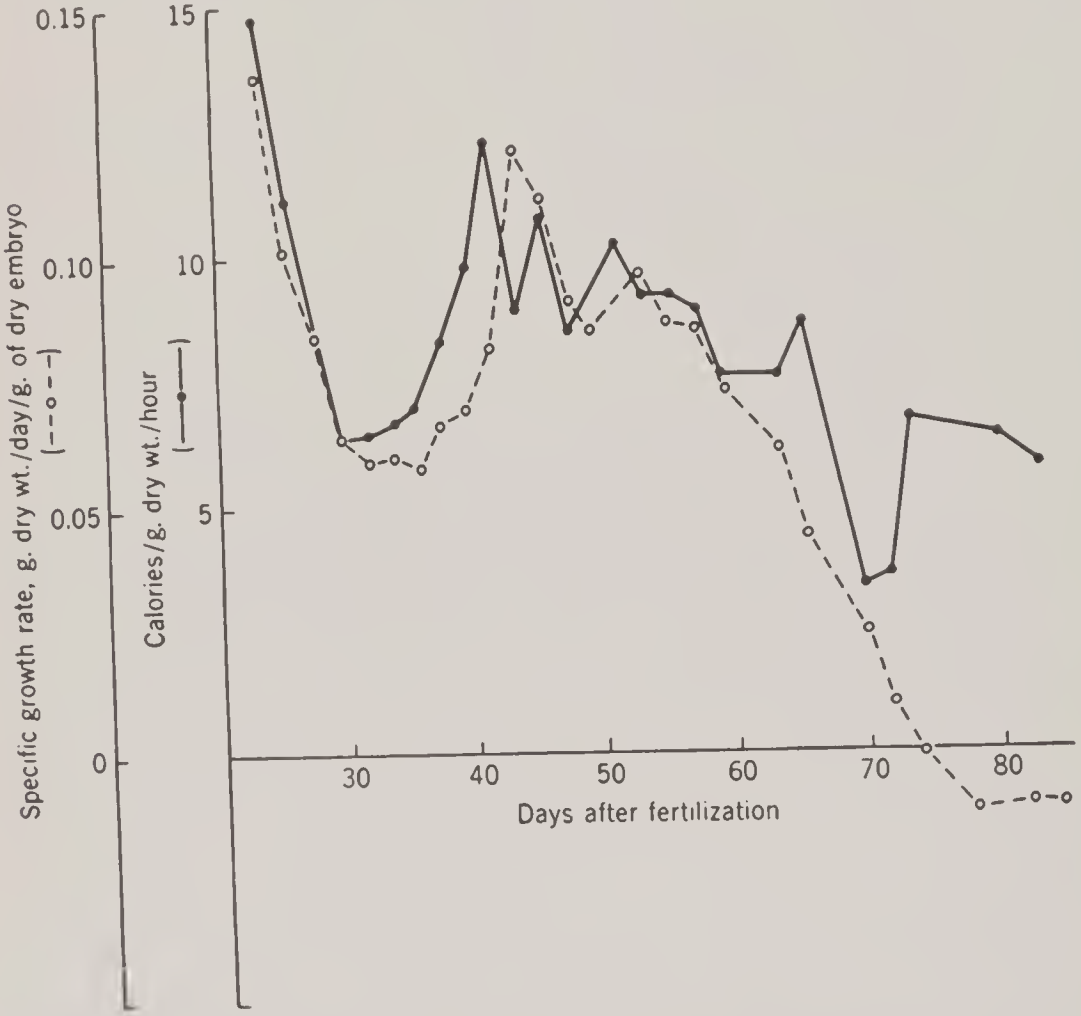

FIG. 1. A comparison of the changes in specific growth rate and specific heat production of rainbow trout (*Salmo irideus*) egg and alevin.

and the rate of growth. High growth rates and high respiration rates occur together. This correlation seems to be true in the rainbow trout, at least from about the 22nd day of incubation through the main phase of alevin growth (Smith, 1956). The values obtained in one series are shown in Fig. 1. The fall from an initially high level in both specific growth rate and heat production per gram dry embryo per hour is more or less simultaneous. After hatching, the rise in both these quantities is not quite so uniform but the general trends in change of the two are

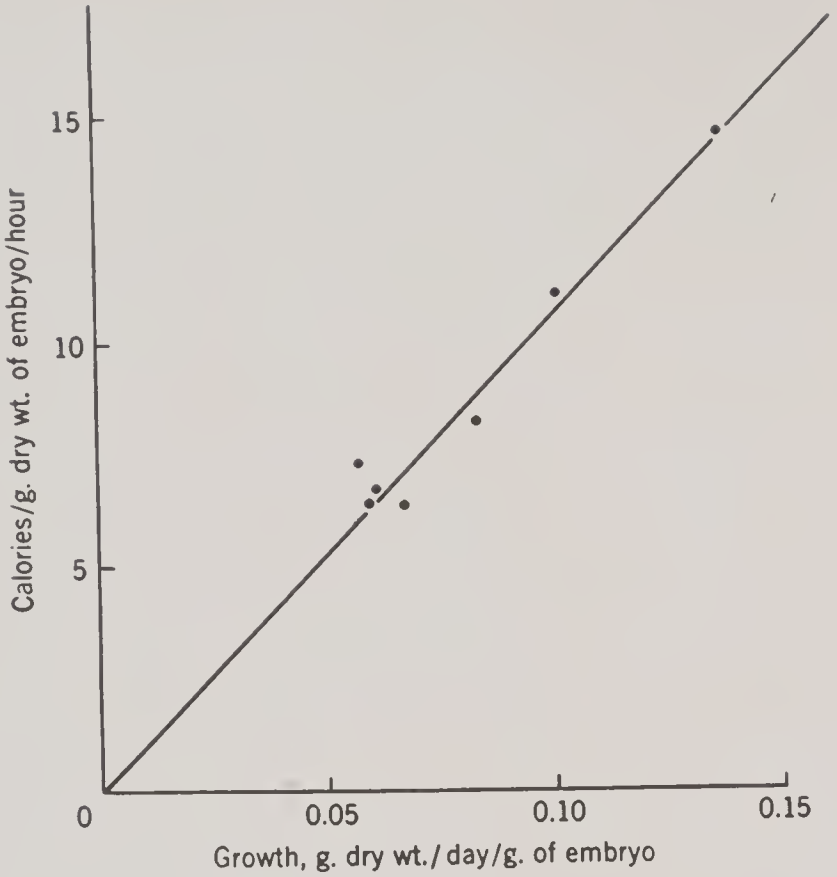

FIG. 2. Specific heat production plotted as ordinate against the corresponding specific growth rate as abscissa are in direct proportion up to hatching.

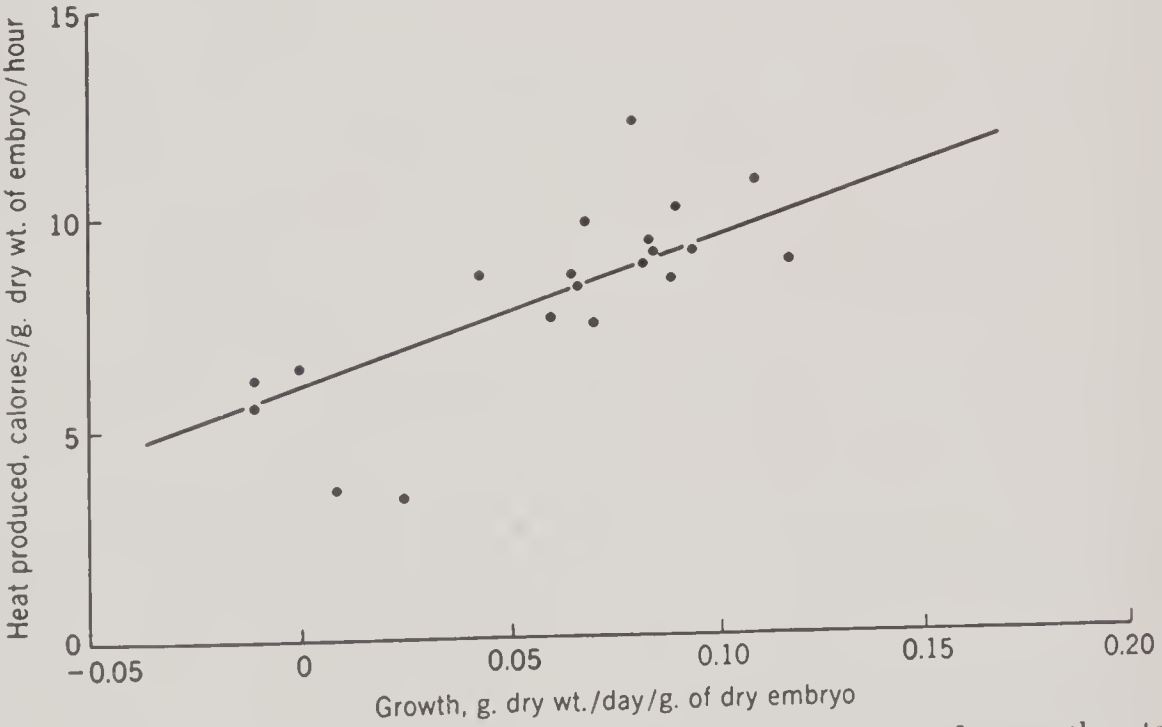

FIG. 3. After hatching the specific heat production and specific growth rate, plotted as ordinate and abscissa respectively, lie around a straight line with positive slope (g) and intercept (m) on the ordinate axis.

clearly similar. This resemblance can be tested further by plotting values of specific heat production against values for the specific growth. Points before hatching lie on a straight line through the origin (Fig. 2), that is to say, specific heat production and specific growth rate are directly proportional. After hatching, the values lie around a line (Fig. 3) of definite positive slope (g), with a positive intercept on the ordinate (m). The equation of the line is:

$$\frac{1}{W_t}(H.P.)_t = m + g\,\frac{1}{W_t}\,\frac{(dW)}{(dt)_t} \qquad (1)$$

Multiplying by W_t we see that:

$$(H.P.)_t = m.W_t + g\,\frac{(dW)}{(dt)_t} \qquad (2)$$

in other words the heat production ($H.P.$) at time t of 100 embryos is the sum of two components. The first term is proportional to the dry weight at this time of 100 embryos (W_t) and is clearly to be associated with maintenance; the second term is proportional to the simultaneous rate of increase in dry weight of the 100 embryos and can be regarded as in some way related to growth. Using the empirical values of the constants m (6.1) and g (35.5), it is possible from the observed values of W_t to predict the heat production at any time; the success can be judged from Fig. 4. The continuous line is the predicted heat production from Equation (2), the points are actual measurements of the rate of heat production. This relationship between maintenance and growth is probably influenced by temperature which may change the relative values of m and g as well as $\frac{dW}{dt}$. Such a mechanism might underlie the variation in efficiency of development with temperature of incubation found by Hayes and Pelluet (1945) for salmon in confirmation of earlier work by Wood and by Gray on the trout.

The fact that a part of the heat production arises from maintenance is nothing new but that there seems a definite fraction relating to the amount of new tissue formed is significant in relation to the accepted views of the dynamic constitution of living tissues. The chemical and energy changes of growing systems can be thought of, in the first instance, as a complex maneuver of small dipoles forced into improbable geometric configuration in relation to pre-existing complex structures, which are stabilized in a dynamic equilibrium by the energy consuming processes of maintenance. The dipoles are held in place by the active expenditure of energy until all parts are assembled and in position for duplicating the pre-existing structure. Thus it can be seen that an energy

dissipation proportional to the rate of growth may be a direct consequence of the nature of the growth process considered as a physical sequence of events. Any complex once established is, of course, thereafter maintained, precisely as the pre-existing complex had been and continues to be, by the exothermic reactions of maintenance which contribute to the $m.W_t$ element of the heat production in Equation (2).

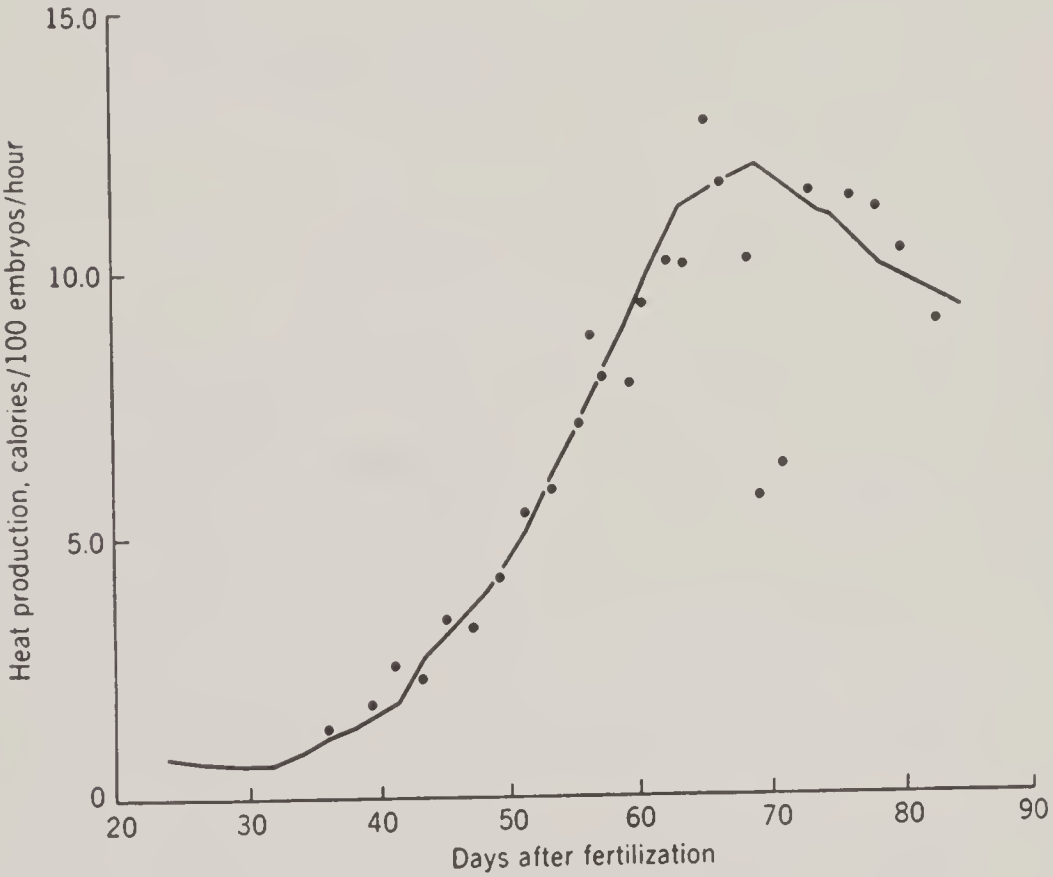

FIG. 4. A comparison of the rates of heat production observed and the values calculated from equation (2).

C. Carbohydrate Metabolism

The ubiquitous distribution of the enzyme systems for glycolysis throughout living tissues (in which fish embryos are no exception) shows that carbohydrate, in all probability, is metabolized in the final steps of energy generation, whether used for mechanical, osmotic, or developmental work. This observation does not get us very far with the problem of carbohydrate metabolism. It is very likely that the carbohydrate used is replaced immediately from some other food source, the general level of carbohydrate of the egg or embryo remaining almost constant though continually metabolized. The quantitative study of carbohydrate stores and their distribution and utilization has mostly been confined to the salmonid egg; information from other fish material

is sparse. Gastaud (1951) gives a preliminary account of the histology of glycogen distribution in *Scyliorhinus* embryos but does not support his observations with quantitative measurements. Shanklin (1954) presents evidence that in later stages of *Fundulus* development glycolysis is concerned in the active maintenance of osmotic differences between the perivitelline fluid and the egg contents. In the salmonid egg, the evidence for carbohydrate metabolism is somewhat indirect. Total carbohydrate or glucose and glycogen have been estimated by chemical and histochemical methods at different stages and the metabolism of these components inferred from the changes in this total amount. Substrates consumed in the synthesis of replacing carbohydrates are regarded chemically as being consumed "tout court," and there is no means of estimating what proportion of daily consumption of protein, for instance, is used for the synthesis of carbohydrate which is itself metabolized directly or else replaces the stored carbohydrates metabolized that day. Another method is to rely upon measurements of the respiratory quotient. Values of 1.0 are assumed to imply that a predominantly carbohydrate substrate is being metabolized. Neither approach is really satisfactory. Even the analytical procedures produce somewhat conflicting results (Smith, 1952). The total quantity of carbohydrate at any time is small and the bulk of yolk itself leads to experimental error. Bound carbohydrate requires release by hydrolysis and it is impossible for this reaction to be carried to completion without gross dilution of the extract for later analysis. Accurate measurement of oxygen consumption and of carbon dioxide output, so needful for reliable respiratory quotient estimation, are also vitiated by bulk of yolk and low turnover rates of gas. Buffering in the perivitelline fluid makes carbon dioxide measurement difficult.

The general picture for the salmonid egg seems to be as follows: it is unlikely that sugar reserves fall to any extent during cleavage; during gastrulation in the salmon, Daniel (1947) claims glycolysis, though Smith (1952) was unable to detect a fall in total carbohydrate in the rainbow trout during the corresponding period. In this fish a marked fall in total carbohydrate occured after gastrulation was complete during the period when the blood circulation was being established. During the hatching period, Hayes and Hollett (1942N) in the salmon, and Smith (1952) in the rainbow trout, both find either interruption in glycogen synthesis in the embryo or a fall in total carbohydrate, possibly arising from consumption of glucose. As the embryo is formed by growth it is generally agreed that it has a small but significant content of glycogen and glucose; at the beginning of starvation these may be metabolized for a short period (Smith, 1952) and this use is clearly a

case of replacement metabolism failing to keep pace with the catabolism.

The respiratory quotient observations do not add anything of value to this picture, they have been reviewed by Hayes (1949) and Smith (1952) whose articles should be consulted.

D. Protein Metabolism

Microanalysis of the nitrogen excreted by the egg and alevin is the most sensitive and direct way of measuring the metabolism of those proteins consumed to maintain the developing embryo. The protein transfers to the embryo are still assessed by direct analysis and the subsequent extraction of as much meaning as possible from relatively small differences between the large figures for the total nitrogen of the embryo. Direct measurements of nitrogen excretion have been made by Smith (1947, 1956) and they show that before hatching there is slight excretion of ammonia only. Other nitrogenous materials are retained in the egg. There is some evidence that the products of digestion of the chorion may be absorbed by the embryo. After hatching the level of nitrogenous excretion and of heat production have been measured on the same material at the same time. The level of protein catabolism is high before, during, and after hatching but then it settles down to a fairly steady low level which is maintained throughout the yolk sac phase of development. Heavy eggs seem more dependent upon protein for maintenance from the 50th to the 67th days than do eggs from the same batch which are 10% lighter in weight. Smith (1956) compares the efficiency of growth for these two batches of eggs in terms of storage and combustion of nitrogen. Storage is greatest in relation to excretion between the 51st and 57th days of development when roughly six times the amount of nitrogen is stored as embryo tissue than is metabolized in maintenance. At other times growth is much less efficient.

Storage and combustion of essential amino acids can be seen from the analyses of Corti (1950); the amount of methionine and phenylalanine destroyed in relation to the amount stored seems much smaller than for other essential amino acids and there can be no doubt that careful selection is occurring in this case. The nonprotein nitrogen in the salmon embryo tissue is approximately one hundredth of the total nitrogen present as protein. It therefore rises in absolute amount during development (Hollett and Hayes, 1946).

E. Fat Metabolism

The metabolism of fats in the eggs of Salmonidae has already been reviewed by Smith (1952). His methods of analysis have been criticized by Lafon (1947) who, quite justly, remarks that much of the argument

depends not upon actual analysis but upon indirect deductions. Smith's approach was to measure dry weight losses, protein breakdown, and heat production. It was clear that carbohydrate combustion could be neglected for all practical purposes. The heat production from protein breakdown was calculated from the nitrogen excretion and also from the dry weight of protein lost by the system. The rest of the measured heat production and the rest of the dry weight lost were attributed to fat metabolism. If this argument be considered sound, then fat metabolism was more pervasive and widespread than the observed combustion of the conspicuous glyceride fat reserves had rendered probable. This particular element is not consumed until the yolk sac phase is nearly at an end, but the observed protein breakdown and observed heat production implied significant fat metabolism during most of the free-living alevin stage. Balance sheets for nitrogen, heat, and total dry materials are given in Smith's papers and the weight of the evidence would seem such that his argument must be accepted for the time being, pending the development of better analytical procedures for the diverse lipid components of the egg yolk complex.

Fat metabolism in the early stages of trout development has been studied by histochemical methods by Mathur (1950). His results are not yet published. Fat droplets in the egg are of two kinds; small lightly pigmented phospholipid and protein, and larger pigmented triglyceride droplets of a high degree of unsaturation and containing some cholesterol. Freezing to —20° C. caused the phospholipid–protein complex to break down to yield droplets of phospholipid. The bulk of the yolk seemed to be a complex of protein and phospholipid bonded in some way by phosphates. This bonding is strengthened by formaldehyde fixation. Lipids move into the vitelline membrane some 24 hours after the egg is laid. Lipid droplets are absorbed into the embryo during gastrular overgrowth, the triglyceride droplets appear at this stage to be inert. In the later stages of development experimentally injected triglycerides could be utilized by the alevin only if they were combined with trout phospholipid. Foreign phospholipid combined with the triglyceride was fatal. The fatty acids of the embryo lipids are more saturated than those of the yolk lipids and the phospholipids of the embryo have associated with them some carbohydrate-containing compound, possibly galactolipid.

In some livebearing teleosts there is evidence that lipid metabolism is of significance as fatty nutritive fluids are a conspicuous feature of the ovarian follicular or epithelial secretions. In elasmobranch gestation there are similar records of the fatty nature of the ovarian food materials.

There is no doubt that fat metabolism is of major importance to the

fish embryo, though it does not have the dominant place in development which is so characteristic of the eggs of terrestrial vertebrates. The ease of disposal of the products of protein breakdown allows fish embryos still to metabolize a high proportion of the yolk proteins.

F. Water and Salt Electrolytes

The trout embryo has a much greater water content than the yolk. Transfer of materials from yolk to embryo entails their dilution and hence water absorption by the alevin. Growth up to hatching is restricted because the chorion does not permit the intake of water sufficient to allow the formation of more embryo. Once access to unlimited water occurs then the rapid phase of growth so characteristic of the recently hatched alevin begins. This account established in essentials by Gray (1926), requires no modification. Hayes and his school have demonstrated the same for salmon development.

The role of salt and electrolyte movements in early development has been discussed in Section II, B and in Section III, C. For the later stages of the development in the salmon the work of Hayes *et al.* (1946) is the most outstanding and useful contribution. They confirm the earlier observations that the depression of freezing point of the yolk contents in the salmon stays constant throughout development. Calcium and sodium ions are absorbed from the environment so effectively that the final amounts are four times and three times the initial amounts, respectively. It is probable that most of the calcium and magnesium of the egg is not osmotically active. There is marked loss of phosphate as development proceeds. Potassium is lost to the environment and appears to be selectively absorbed by the embryo from the yolk.

V. DEVELOPMENTAL MECHANICS OF THE FISH EGG

The reaction of fish egg material to experimental interference has only received sporadic study during the present century. The most notable progress has been due to the work of few authors and has been significantly advanced since 1930 by the development of artificial media and the techniques of sterile culture associated with the name of Holtfreter. The earlier work of Luther and Oppenheimer has been summarized and reviewed by Oppenheimer (1947). Since then Devilliers and his coworkers in France, and Trinkaus in the United States, have added much to our knowledge. There is a large Russian literature notably on the eggs of various sturgeons; this has been the subject of a recent comprehensive book (in Russian) by Detlaf and Ginsburg (1954). Their outlook is both that of descriptive morphology and of experimental embryology and the illustrations are of high quality. The recent review of

experimental embryology of teleosts by Rudnick though not published until 1955 appears to have been drafted early in 1952. This section will accordingly include a rather more detailed evaluation of the more recent work.

It perhaps needs emphasizing that, even in teleost eggs, there is a considerable degree of variability, so that observations on one species should not be considered to have a more general validity. It seems to be true that the size of the egg has a direct influence on the capacity of an egg to resist without damage the effect of periods of anaerobiosis and the conditions essential for the initiation of gastrulation seem to depend upon the material studied. Egg size may influence the capacity for self-differentiation of the blastoderm when cultured in isolation in a saline medium, for the trout germ, though continuing to live when isolated, forms only a mass of cells, whereas the germ of a pike egg under the same conditions will self-differentiate into a small embryo (Devilliers, 1947).

There appears to be no constant relationship between the embryonic axis and the plane of the first cleavage, though in general there seems to be a tendency for the plane of the second cleavage to coincide with the long axis of the embryo. The early cleavage stages of teleosts show considerable powers of regulation and the early cleavage blastomeres in *Fundulus* have been claimed totipotent (Nicholas and Oppenheimer, 1942). Out of 72 cases where one of the blastomeres at the two-cell stage was removed, 65 embryos of small size but normal structure were formed. This does not seem to be the case for the goldfish *Carassius* (Tung *et al.*, 1945) in which the same operation gave only 55% of cases forming an embryo, the rest showing only highly abnormal forms or mere vesicular structures. Shaver (1953) has succeeded in dividing the blastomeres of *Fundulus* egg at the two-cell stage while retaining some yolk attached; about one-half to two-thirds of the yolk is lost during the operation. Out of 200 successful operations 50 pairs of fragments lived to cleave and to show partial or even complete gastrulation. No embryonic axis was found in any instance; however, when two blastomeres were separated from the rest of the egg at the four-cell stage, some preparations showed indications of tail structures. Blood and pigment cells do not appear to be axial in origin in the case of *Fundulus* for they frequently developed at the yolk sac surface in the absence of all other embryonic structures.

Luther (1934–1939N) operating on *Salmo* material at an early gastrula stage, removed the extraembryonic half of the germ and cultured it on the yolk sac epithelium of an older host. Under such conditions, whole embryos would develop but the possibility of the host influencing the

graft should be recognized. Devilliers (1955) has recently expressed the view that his studies of salmonid material make it clear that material which is located on the edge of (or beyond) the blastodermic vesicle at the late segmentation stage and which is later overgrown by blastodermic material in the early stages of gastrulation is essential for the normal development of the embryonic axis. If this view is accepted, then the differences quoted above in the behavior of parts of eggs might be a consequence of differences in timing the blastoderm separation, the degree of bipolar differentiation, and of incorporation of axial determining material into the early cleaving blastomeres.

Twinning and axial duplications become apparent at gastrulation and here the operation of an "organizer" can reasonably be inferred. The regional properties of the invaginating chorda mesoderm material in salmonids was long ago demonstrated by Eakin (1939N). There is some evidence that the material of the organizer may be shifted relatively to other structures by centrifugation (Shaver, 1951). Forsthoefel (1951) centrifuged the eggs of *Fundulus* some 10 to 30 minutes after fertilization at the time of the first cleavage. He got three cases of twinning out of 510 eggs. Centrifugation of older blastoderms produced considerable disturbance of the material in the germ ring leading to abnormalities in the invaginating chordamesoderm material. Shaver and Ito (1952) attempted to enhance the centrifugation effect by combining it with low temperature treatment (4 to 6° C. for 24 hours). They did not succeed but reported a 20% increase in mortality. Barnes (1953) in a more extended study confirms that the period of maximum sensitivity to centrifugal forces is within the 30 minutes following fertilization. The resulting abnormalities, (defective head structures, mesodermal deficiencies, and rudimentary parasitic twin structures) are all consistent with the view that some organizer substance has been moved by centrifugal force relative to other parts of the germ. It is, however, possible that some of the abortive neural differentiations might arise in blastoderms from accidental local cytolysis rather than a displacement of normal organizer material. Oppenheimer (1950), working on *Fundulus,* reports that operations on the gastrula stage designed to increase the amount of neural material at the level of the host medulla show that the embryo has a most variable capacity for functional regulation. Frequently structures of a non-nervous type were differentiated or induced by or near to the grafts, producing a mechanical block with consequent morphological abnormalities. In some instances duplications of parts of the host brain occurred but correlation of neurological structure and behavior was difficult.

Recently the mechanics of teleost gastrulation has received a good

deal of attention. For salmonid material Devilliers (1951, 1952) considers that the main agent involved in gastrulation is the superficial coat of the germ and of the attached perivitelline pellicle; the latter is supposed to perform an active contraction during gastrulation. The expansion of the cellular germ with its portion of the superficial coat is a necessary component of the epibolic movements. If the blastodisc be removed entirely a contraction of the underlying yolk syncytium draws the edges of the yolk pellicle together and restores the integrity of the envelope of the yolk sphere, but it is only in such a gross operation that the yolk syncytium plays an active part. Neither syncytium nor pellicle shows the least sign of epiboly though the egg, if kept in running water, "lives" for several days after the operation. If a portion of the surface layer of the embryo at the morula stage is scraped off leaving only the deeper blastomeres, the perivitelline pellicle recovers the stripped surface. The healed blastodisc does not show any subsequent epiboly. The reverse operation of removing some of the lower blastomeres without significant damage to the surface pellicle has no effect on the subsequent epiboly. The extraembryonic half of the germ is responsible for the major part of the epiboly as can be seen from the following experiments. If the extraembryonic half of one germ is exchanged for the embryonic half of another giving two extraembryonic halves in one case, these spread over the yolk exactly as in an unoperated egg. The two embryonic halves together only succeed in reaching one-third the diameter of the controls in the same time. The recent marking experiments of Brummett (1954) would indicate that in the case of *Fundulus* the extraembryonic area is likewise of great importance for epiboly. The work of Trinkaus (1951) shows that in this genus the mechanics of gastrulation resemble the trout quite closely in spite of a considerable difference in egg size.

The action on the trout egg of markedly hypertonic solutions, of $\Delta = -1.52$ (the egg content has $\Delta = -0.5$), causes arrest at the morula stage (Devilliers, 1950). If the solution is applied during gastrula overgrowth, the epiboly appears to go into reverse and the germ contracts to a ball resembling a morula. If the salt treatment is applied for only 4 hours and the eggs are then returned to running water, they resume gastrulation and after 26 hours are at the same stage as the control. Detergents produce the same effect. The hypertonic solutions, with large concentrations of monovalent cations, seem partially to liquefy the perivitelline pellicle which is in consequence unable to maintain the regular tension for normal gastrular movement. Elegant transplantations between eggs from the same female of the pellicle from a blastoderm in which epiboly had not yet begun (reared at 4° C.) and an equal area of the center of a blastodisc nearing the end of gastrulation (reared at 12° C.)

showed that the tissue of more advanced development transplanted into the earlier stage formed a permanent vesicular outgrowth, epiboly having been blocked in the implant. Less advanced tissue in the later embryonic ring showed epiboly which was normal but slightly accelerated in relation to the control. Devilliers (1952) concludes that extension of the germ and contraction of that part of the pellicle over the yolk are of equal importance for epiboly, but the whole continuous superficial complex of germ and pellicle constitutes a single functional unit.

The dumbbell shape assumed by the egg of *Gobius paganellus* (Oppenheimer, 1954) in the middle of gastrular overgrowth is very difficult to fit in with any simple scheme of surface contractions and sol–gel transformations.

The action of lithium on *Plecoglossus* eggs (Ogi, 1951) seems in every way comparable to the results obtained on amphibian material.

Lopashov (1944) has studied the origin of pigment cells and visceral cartilages in teleosts using loach (*Misgurnus fossilis*) and perch material. Parts of the early mid-dorsal region of the brain rudiment (in which at this stage the neural crest material is still incorporated) were extirpated or implanted and while some degree of regeneration replaced material after removal, both the pigment cells and the cartilages of the splanchnocranium arose from neural crest, thus confirming what has been found already in amphibian material. There was considerable pigment cell growth and some cartilage arose at sites of implantation under the blastoderm of the gastrula well away from the host embryonic axis; in one anomalous case, the same complex developed from an implant of invaginated axial mesoderm!

Trinkaus (1952) finds that after excision of the entire embryonic shield in gastrula stages of *Fundulus,* leaving extraembryonic material only which spreads to close the wound, pigment cells appear later in these specimens. This would suggest an extraembryonic origin for this material in *Fundulus.*

The later organogenesis of fins has been studied by operative techniques by Blanc (1951) and François (1955).

The topographical distribution of presumptive materials in elasmobranch embryos was investigated by Vandebrock (1936N) by the application of vital dyes to early blastoderms. Kopsch (1949) has made a special study of the early history of the presumptive chorda and mesoderm in two species of elasmobranch fish. The lengthening of the body and tail did not occur by differentiation from hitherto indifferentiated tail bud material but by growth of morphologically distinct elements of each of the three germ layers. The origin of the posterior body and tail structures in *Fundulus* has been traced by carbon marking techniques

by Brummett (1954) whose results are a useful clarification of the extent to which material from the germ ring is incorporated into the fish embryo body. Most of the material of the anterior half of the germ ring clearly forms surface elements of the yolk sac only and takes no part in the formation of the embryo body.

VI. HATCHING

The onset of hatching in the teleost egg is accompanied by a complex of phenomena, the interrelations of which are not easy to discern. Hatching involves the secretion of a proteolytic hatching enzyme from specialized glands probably of endodermal origin (Ishida, 1944) with a distribution characteristic of the species. The secretion and activity of this enzyme is sensitive to external conditions, notably temperature (Gray, 1926; Hayes, 1942); it is moreover, relatively rapid in action, as seen from studies of dry weight of the shell, changes in egg resistance to applied pressure, and similar observations of mechanical properties. Wu and Wang (1948) report in the eggs of *Macropodus opercularis* and *M. chinensis* the secretion of an additional enzyme from mucous glands in the fish which inhibits the digestion of blood fibrin *in vitro* by ordinary proteolytic enzymes. The secretion of this anti-enzyme persists for as long as twelve hours after the hatching of the fish.

In the trout egg (Smith, 1947) the normal activity of the hatching enzyme leads to the perivitelline fluid becoming extremely thick and viscous. It contains at that time a great quantity of compounds containing amino-nitrogen; these are retained within the chorion and may be in part resorbed by the young fish. Hayes (1942) reports a sharp rise in primary amino-nitrogen content of the privitelline fluid of the salmon egg at hatching. While it is difficult to make precise measurements, a careful study of the total nitrogen content of eggs, selected both for uniformity of size and similarity in stage of development, revealed that of the nitrogen in the chorion of the newly laid egg only about 20% was lost at hatching as thin fragile membrane or as material in solution. It seems highly probable that the products of digestion of the chorion are to a large extent resorbed by the alevin shortly before hatching.

In the salmonid egg, the water content of the embryo appears to fall slightly before hatching, to rise again when unimpeded access to water is restored after hatching. At the same time there is seen a decline in the rate of growth (Priwolnev, 1938; Smith, 1956) accompanied by a fall in the specific rate of oxygen consumption and of heat production. Hatching is thus a momentous event in the life of the alevin. It is all the more difficult therefore to comprehend the extremely wide variations in the timing of this event between related species. For instance, at

10° C. *Salmo salar* hatches after about 68 days of incubation, *Salmo irideus* after 35 days only. Both species will tolerate experimental procedures designed to accelerate hatching, the most effective being to raise for a short while the temperature of incubation. Hayes (1942) reports for *Salmo salar* an optimum of 21° C. for the hatching enzyme. It is of interest that the behavior of hatching enzymes in the sturgeon, a primitive chondrostean, is similar to that found in teleosts. Zotin (1954) has studied the effect of temperature on the accumulation of the hatching ferment both in the perivitelline fluid and in the embryo tissues. The effect of the enzyme on the toughness of the egg shell was also followed. He also gives figures showing the morphological distribution of the sites of secretion over the head and anterior third of the yolk sac both in the sturgeon and salmon embryo.

The protein of the chorion of the salmon egg has been analyzed by Young and Inman (1938N). The casing resists digestion *in vitro* by trypsin, and pepsin has only slight action. The chorion appears to be a pseudo-keratin and its solution by the hatching enzyme (Hayes, 1942) is not considered a true hydrolysis because digestion results in such a small yield of free amino acids. Ishida (1944) finds that the hatching enzyme of *Oryzias latipes* works at the relatively alkaline optimum pH of 8.0–9.0, being inhibited by 0.02 *N* KCN and 0.04 *N* H_2S. This suggests that the natural enzyme is a tryptase and in this species the chorion may be dissolved *in vitro* by pancreas extracts and by preparations of commercial trypsin. The chemical identity of teleost hatching enzymes clearly requires further study.

It must be recognized that the activity of the embryo is probably of great importance for hatching. Ishida (1944) reports in *Oryzias* that the initiation of respiratory movements, especially of the operculum, appears to rupture the unicellular (18 μ diameter) hatching glands of endodermal origin which line the pharynx. Digestion of the shell and hatching follow shortly after. The importance of respiratory movements for initiating hatching might underlie the observation of Trifonova (1937) that hatching can be advanced by as much as 5 to 7 days in salmon eggs if they are subjected to some 6 hours of asphyxia by bubbling hydrogen through the hatchery water. Milkman (1954) notes the converse effect in *Fundulus;* hatching can be delayed indefinitely by maintaining a high oxygen tension in the sea water in which the eggs are kept. This inhibition by oxygenated sea water is destroyed by boiling, but not by heating to 65° C. After this oxygen-induced inhibition, if the eggs are washed in normal sea water, they hatch immediately. The sequence of events is as follows: first, activity of the mouth, then excretion of granular material from the anus, and contraction of the yolk sac to one third of its former

volume. After this the experimental application of oxygenated water no longer leads to delay in hatching. Some 7½ minutes later and after 10 intrachorional somersaults the embryo breaks out. Of the original smooth, tough, and relatively thick chorion, only the outer of the two layers is left, now roughened, thin, and flaccid.

It is perhaps not wise to assume that every glandular structure appearing about the time of hatching is significant for hatching; Brinley and Eulberg (1953), for instance, describe in the cichlid, *Aequidens portalegrensis*, six spherical, relatively large (200 μ diameter), mucus secreting glands on the head. The secretion from these is not enzymatic and appears to allow adhesion of the newly hatched young to stones.

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Chapter IX

EXPERIMENTAL STUDIES ON GROWTH

M. E. Brown

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I. INTRODUCTION

Since fish form an important source of human food, the production of fish flesh under different natural and artificial conditions is of considerable economic interest. To be able to forecast the amount of fish which would be produced in any body of water in a given time would be very valuable but fishery biologists cannot yet achieve the accuracy of agricultural experts in forecasting crops.

There are a great many published studies of individual fisheries and these usually include details of the catch in successive years with the size distribution (length or weight or both) and sometimes the age distribution, as calculated either by the Petersen method or from

examination of scales, otoliths, or bones. Some account of the use of scales in age and growth determination is given in Chap. V; rings on otoliths, opercular bones, vertebrae, or fin-rays have been used in a similar way for certain species of fishes. This information about the size and age attained by various species in many different waters is subject to sampling errors (variation in fishing effort, etc.) and also perhaps to errors in interpreting and measuring growth zones and annual rings. Analysis of long series of records for a given water has sometimes made it possible to establish a correlation between the catch and other recorded phenomena and thus to forecast probable catches in succeeding years.

Comparison of records from different waters often suggests that differences in number of individuals and in their growth may be correlated with varying physico-chemical and biotic factors or perhaps with genetical differences between fishes of the same species. It is, however, exceedingly difficult to establish convincing correlations from which to predict the effect of a single factor on the growth of an individual fish. Such definite correlations can be established only under the controlled conditions possible in a laboratory; it is with such studies that this review is mainly concerned but they must be supplemented with information from studies in the field.

Only when all other possible factors have been "controlled" or measured during the course of an experiment can the effect of any single factor be observed clearly, and this situation has not often been achieved. If fishes have lived in aquaria at room temperature and in natural daylight, it is difficult to assess the effects of the varying temperature and light even if both have been recorded daily. If all the fishes have been weighed as a group and some individuals have died during the experiments, the change in average weight may be very misleading if the fishes which died were either larger or smaller than average. Ideally, records should be kept for each individual fish and the following physico-chemical factors should be held as constant as possible: temperature, amount and intensity of illumination, volume and shape of aquarium tanks, degree of crowding of fishes, rate of water flow (if any) and chemical composition of the water, including gases in solution as well as salts and "trace elements." The fish should be fed at regular times on food of constant quality and the routine of cleaning the tanks and of weighing and measuring should be carried out on a regular schedule. The fish should be of known "history" (heredity, age, previous treatment, etc.). Under these stringent conditions, reproducible results should be obtained. By suitable manipulation of the experimental conditions, the effects of the various factors can be established.

II. THEORETICAL CONSIDERATIONS

A. Growth Rates

Minot (1908) was the first person to state simple theoretical principles relating size to age in animals. There have been numerous attempts to devise fairly complex mathematical formulae to fit growth curves or to predict curves from theoretical principles, but these are of limited application and hence of less general interest. Critical discussions of such formulae may be found in Gray (1928) and Brody (1945). Medawar (1945) based his discussion of growth functions largely on Minot's ideas and stated five "laws of growth;" these describe mammalian growth but can be applied to the growth of fishes only with certain qualifications.

Medawar's first law of growth, "size is a monotonic increasing function of age," implies that animals always grow bigger. This is true of fishes if growth is measured in terms of length; there may be periods in which there is no increase in length but there can be no decrease, except in rare cases of metamorphosis such as the change from leptocephalus to elver of the eel, *Anguilla*. Fishes regularly lose weight; this is most obvious during the breeding season when female trout, *Salmo trutta*, for instance, may lose 15% of their "ripe" weight. Adverse conditions and starvation may lead to loss of weight; Swift (1955) found that trout in a hatchery lost weight in January and February although they were given plenty of food. Thus, detailed growth curves for length and for weight of fishes may not be identical in shape and it is important when planning experiments to decide which of these measurements to record though often both may be of interest.

Medawar's second law states: "What results from biological growth is itself, typically, capable of growing." This is true of most of the tissues of fishes though some, e.g. bones and scales, grow only by addition of material outside them. Since most of the newly formed tissue itself begins to grow as soon as it is formed, the process of growth in fishes is multiplicative. This is recognized in growth equations by using logarithmic expressions of the type necessary for calculating "continuous compound interests." Equations of the type used in simple interest calculations do not express multiplicative growth adequately.

Medawar's third law applies only to organisms when the external and internal environments remain constant and most animals, including fishes, should conform instead to the fourth law of growth: "under the actual conditions of development, the specific acceleration of growth is always negative." However, this law can be applied to fish growth only with considerable qualifications since the majority of fishes show cyclical changes in growth rate with alternating periods of rapid and

slow growth. If, however, growth rates are calculated for the same fish at the same stage of successive growth cycles, these rates generally decrease as the fish grows older. Most fishes living under temperate conditions have annual cycles of growth and the average annual specific growth rates of these fishes typically decrease with age. Since most of the available data from temperate natural waters consists of sizes calculated for time intervals of one year (e.g. from rings on scales), the specific growth rates derived from these data should conform to Medawar's fourth law. Exceptions may occur when the fishes change their environment or their food; thus salmon, *Salmo salar,* grow more rapidly in their first year in the sea than in the preceding year spent in fresh waters.

"The specific growth rate declines more and more slowly as the organism increases in age" is Medawar's fifth law, and, again, it does not always apply to fishes although it is generally true for specific growth rates calculated for complete growth cycles. Thus, it generally applies to rates derived from the estimated annual sizes of wild, temperate fish. Minot was the first person to recognize that for most animals the specific growth rate is highest early in life and that it typically decreases with increasing age, becoming zero for some animals. He considered the decrease in growth rate to be a measure of senescence and his epigram, "organisms age fastest when they are young," is expressed by Medawar's fifth law.

Medawar considers that the fundamental generalization about the relationship between size and age for an organism can be expressed by the equation:

$$\frac{d \log_e Y}{dt} = K.f(t)$$

where Y is the size, $K.f(t)$ is a positive quantity such that $f(t)$ decreases with t (time) and $df(t)/dt$ increases with t towards zero. This may be true for most fishes when the periods of time considered are sufficiently long (i.e. periods of twelve months for temperate fishes) but it may not hold for growth over short periods. Thus, if Y represents weight, $K.f(t)$ may become negative and even when Y is length, $f(t)$ may increase with t, e.g. when a salmon smolt reaches the sea or when trout begin to grow faster in spring after little or no winter growth.

The shapes of growth curves for fishes may vary considerably according to the degree of detail in the information available. However, if growth cycles are "smoothed out," e.g. by using annual data for temperate fish which have annual growth cycles, curves showing lengths or weights plotted against age are generally sigmoid; the arithmetic growth

rate (dY/dt) is thus maximum in the middle of life and its acceleration (d^2Y/dt^2) is first positive and then negative. For the same data, logarithms of length or weight plotted against age give smooth curves which rise asymptotically; the specific (multiplicative) growth rate ($d\log_e Y/dt$) decreases continuously through life but with decreasing acceleration ($d^2\log_e Y/dt^2$). For individual fishes, however, or for species such as *Salmo salar,* even smoothed growth curves may diverge from these typical ones.

Walford (1946) has shown that when the length at age $n+1$ is plotted against the length at age n, the data for many animals, including some fishes, form straight lines for ages after the point of inflection of the sigmoid growth curve. The slopes of these lines measure the deceleration of the specific growth rates and Walford suggests that such slopes may be characteristic of races within a species and could thus be used to differentiate between them. Felin (1951) found that data for individual *Platypoecilus maculatus* of one family, grown at two different temperatures, fitted the same line and thus had the same slope although the fish differed considerably in size.

The specific growth rate (g or G) can be calculated easily from the following equations:

$$Y_T = Y_t \cdot e^{g(T-t)}, \text{ or } G = 100g = 100. \frac{\log_e Y_T - \log_e Y_t}{T - t}$$

where Y_T and Y_t are the sizes recorded at times T and t, T being later than t. G is expressed as per cent per unit time. G is likely to vary in time and probably to decrease generally with age (Medawar's fourth law) so the greater the time interval $T-t$, the less accurate is the calculation. Ideally, the time interval between measurements should be as short as possible, especially early in life when values of G are highest and are changing most rapidly. Since the calculation takes account of both initial and final sizes, G can be used to compare the growth of animals of markedly different sizes.

B. Growth Patterns of Fishes

Fish are poikilothermous animals living in water and for most of them growth is a continuous process, though generally with fluctuating velocity. Mammals and birds are homoiothermous animals living generally on land or in the air; their life history can generally be divided into an embryonic period (passed *in utero* or in the egg), a juvenile period, an adult period (during which the animal can reproduce), and finally a senile period. Growth in size occurs during the embryonic and juvenile periods but the adult animal generally does not grow larger and the

senile animal may even shrink and shows progressive loss of faculties. Fishes, also, have an embryonic period (discussed in Chap. VIII) but generally it is difficult to distinguish juvenile, adult, and senile periods. As soon as the young fish begins to feed and becomes independent of its yolk supply, it starts a period of growth which may continue for the rest of its life—although the specific growth rate may fluctuate and on the average declines progessively. At some stage, the fish becomes sexually mature and spawns and subsequently it may spawn at regular intervals for the rest of its life. Between reproductive periods, however, the fish may continue to grow larger until eventually it dies. Thus there may be a considerable range in size among sexually mature fishes of the same species.

Bidder (1932) discussed the contrast between the characteristic growth patterns of mammals and fishes and pointed out that, whereas an adult mammal is continually in greater danger of competition from a younger, stronger adult of the same species, a fish as it grows older is progressively in less danger from younger, smaller "adults." The size of terrestrial and flying animals may be limited by their need to maintain a ratio between the weight of the body and the cross-sectional area of the supporting limbs. Aquatic animals live in a medium of higher specific gravity than air and have no mechanical limits imposed on their potential maximum size. It is perhaps significant that whales, wholly aquatic animals, have growth patterns unlike those of typical mammals and continue to grow after they have attained sexual maturity and probably throughout their lives.

Although many fish continue to grow after attaining sexual maturity, this event almost always marks a change in growth pattern; subsequent average growth rates are generally lower than those of immature fishes of the same age because a considerable amount of the food absorbed is transformed into sperm and ova instead of into muscle, bone, etc. Exceptions to this general rule are the male salmon "parr," *Salmo salar*, which often spawn before migrating to the sea; their growth when they reach the sea is nevertheless more rapid than during their last year in fresh water. Some species of fish spawn only once and then die, e.g. *Oncorhynchus*, *Anguilla*, and these are fish which undertake considerable migrations; other migratory fish, e.g. *Salmo salar*, may repeat their journeys several times and may feed and grow between each spawning migration. Many fishes breed frequently at short intervals of time and these may cease to grow, almost or entirely, as soon as they become sexually mature. Many tropical fishes have this "mammalian" type of growth pattern, for their breeding activities are not dependent on environmental factors which vary in an annual cycle. In some small tropical

fishes, e.g. *Heterandria,* both sexes stop growing and thus attain a "specific size;" in others, e.g. *Xiphophorus, Lebistes,* the males attain a specific size while the females continue to grow after maturing. Most fishes living in temperate or arctic conditions breed at definite seasons, probably because their spawning cycles are controlled by environmental factors (see Chap. VII), and after maturity they breed annually or every two or more years.

Since the average specific growth rate decreases after the onset of sexual maturity in most fishes, perhaps even to zero in some fishes, the factors responsible are of interest to those who wish to produce large fish. The age of first spawning might be constant for a given race or species or it might depend on the previous growth history of the individual, sexual maturity being attained at a definite length. Alm (1949) has shown that for two races of Swedish trout, *Salmo trutta,* sexual maturity depends on age and the age of first spawning is inherited (see p. 377 and Vol. II, Chap. X). In the salmon, *Salmo salar,* however, the age at which smolts migrate to the sea depends on how fast they have grown; the majority migrate at three years, the largest two-year-old fish migrate and some, the smallest of their year group, remain in fresh water until they are four years old. The age at which female salmon return to spawn for the first time varies, those which grow faster and migrate earlier return when younger than those which grow more slowly and migrate later. In East African waters, the sizes of mature individuals of *Tilapia* species vary (Lowe (McConnell), 1955); these grow little after attaining sexual maturity and there is some evidence that those exposed to a greater amount of light breed earlier and at a considerably smaller size than those living in conditions of less illumination. There is certainly great variation in the age at which *Tilapia zillii* may begin to spawn; individuals have lived for two years in aquaria, growing slowly without maturing, whereas in ponds they may mature in six weeks when smaller than the immature fish in the aquaria. European eels, *Anguilla anguilla,* differ, even in a single river basin, in the age and size at which they migrate downstream; the factors which initiate this spawning migration of individual eels are not known but individuals prevented from migrating have survived without becoming sexually mature for more than 50 years. Thus, while the onset of sexual maturity is an important event in the growing life of a fish, the factors which control this must be different for different species and have scarcely been investigated.

The causes of death of fishes include disease, attacks by predators, adverse physico-chemical conditions, and failure of the food supply; it is possible that individuals of some species might die from "senescence"

TABLE I

THE LONGEVITY OF FISHES IN CAPTIVITY [a]

Species	Usual Longevity of Medium-Sized Fish (years)	Maximum Observed Longevity (years)
Silurus glanis	–	60 and more
Anguilla anguilla	7–11	55
Anguilla rostrata	–	50 probably
Cyprinus carpio	9–15	47
Acipenser ruthenus	25	46 and more
Polypterus senegalus	5	34 and more
Synodontis schall	–	31 and more
Hippoglossus hippoglossus	–	30 and more 40 possibly
Leuciscus idus	–	30
Carassius auratus	6–7	30
Marcusenius isidori	–	29
Pleuronectes platessa	–	25
Lepisosteus osseus	–	24
Amia calva	–	24
Roccus lineatus	13–14	24
Epinephelus gigas	–	23 and more
Misgurnus fossilis	–	22 and more
Dasyatis pastinaca	–	21
Raja clavata	–	20
Epiceratodus forsteri	–	20
Clupea harengus	9	18–19
Salvelinus malma	–	18–19
Salmo trutta	6–8	18 and more
Scyliorhinus stellaris	7	18 and more
Protopterus annectens	10	18
Saccobranchus fossilis	–	18
Abramis brama	8–10	17–18
Bagrus bayad	–	17 and more
Clarias lazera	–	16 and more
Loricaria parva	–	16 and more
Barbus bynni	–	16 and more
Spondyliosoma cantharus	–	15 and more
Stizostedion vitreum	–	15
Lucioperca lucioperca	–	14
Micropterus dolomieu	10	14
Gadus aeglefinus	8	14–15
Gadus virens	12	14
Gadus morrhua	8	13
Perca flavescens	6–9	13
Salmo salar	8	13
Coregonus clupeiformis	8	12

TABLE I (*Continued*)
THE LONGEVITY OF FISHES IN CAPTIVITY [a]

Species	Usual Longevity of Medium-Sized Fish (years)	Maximum Observed Longevity (years)
Esox lucius	7	10 and more
Perca fluviatilis	7–9	10
Scyliorhinus canicula	7	8
Sardina pilchardus	5	8
Macropodus opercularis	–	8
Aequidens tetramerus	–	7 and more
Cichlosoma nigrofasciatum	–	7 and more
Geophagus brasiliensis	–	7 and more
Tetraodon fahaka	–	6
Hemigrammus unilineatus	–	5
Blennius pholis	–	4
Hippocampus hudsonicus	–	4
Tilapia nilotica	–	4
Gobius minutus	–	2
Aphia pellucida	–	1

[a] From Bourlière (1946), mainly from Flower (1925, 1935).

(Comfort, 1956) but that others are potentially immortal. For many food fishes, man is the most important predator on the larger individuals and his activities may ensure that very few are able to survive beyond a certain age. The mortality rate for young fishes is generally very high and it is probable that the expectation of life increases with age until the fishes reach the minimum size for capture by man.

Fishery studies have established the maximum ages of fishes caught in various waters; these vary considerably as do the average ages for the waters and sometimes the variations can be explained in terms of environmental factors. It is often difficult to assess accurately the age of old, wild fish for, as the specific growth rate decreases with age, the marks on scales or bony structures become less widely separated and indistinct. It is likely that records for longevity will come from aquaria or fish ponds where the fish are fed regularly and protected from adverse conditions. Some such records are listed in Table I. Many small aquarium fishes are annuals, e.g. *Aphia pellucida*, others usually live for two or three years only, e.g. *Lebistes*, *Xiphophorus*, *Betta pugnax*. "Life tables," comparable with actuarial data for man, are not yet available even for these short-lived species but are being prepared for *Lebistes* by Comfort. Statements that carp, *Cyprinus carpio*, may live in ponds up to 170, 200, 300, or even 400 years are probably grossly exaggerated.

Comfort (1956) discusses the incidence of senescence among animals. In mammals, senescence is associated with loss of reproductive capacity and characteristic symptoms of "old age." Some fish have a limited reproductive life; goldfish, *Carassius auratus,* grown in aquaria, usually have maximum fertility at about 3 years of age and become sterile when about 7 years old although they commonly live for more than 17 years and may attain 30 to 40 years. When they have ceased to be fertile, goldfish usually become less sensitive to adverse conditions and become fatter. In the bream, *Abramis brama,* the gonads mature at 3 years of age and regress at about 6 years although the fish continue to grow. The specific growth rate decreases throughout life but its rate of decrease changes at the onset of sexual maturity and changes again after regression of the gonads; this final steady rate of decrease of specific growth rate was maintained until the observed fishes were 13 years old. The sturgeon, *Acipenser stellatus,* however, maintained a steady rate of decrease in annual specific growth rate throughout life (observed up to 30 years) with no obvious change when the fish became sexually mature (at about 15 years of age).

Thus, some fishes seem to undergo senile changes and may have a life span which would be terminated by senescence under the protected conditions of an aquarium whereas other fishes may have an indeterminate life span under ideal conditions. There is great need for more observations of individual life spans and the effects on these of environmental factors.

C. The Relation Between Length and Weight

The relation between length (L) and weight (W) of a fish can be expressed by the formula:

$$W = a.L^n,$$

where a is a constant and n an exponent lying between 2.5 and 4.0. The accurate calculation of a and n involves collecting large numbers of records of W and L for individuals, the construction of graphs on logarithmic paper followed by the calculation of regression coefficients, and statistical analysis of the significance of apparent differences between groups of fishes. This has not been achieved very often. Le Cren (1951) has recently discussed the length–weight relationship with special reference to the perch, *Perca fluviatilis,* in Windermere.

The exponent, n, is the ratio of the specific growth rates for length and weight; where there is no change of shape during growth, $n = 3.0$, as for *Salmo trutta* in Windermere. Often, n may differ for fish of the same species from different localities or it may be different for immature and mature fish even when there is no obvious metamorphosis. Le Cren

classified his perch into six groups and found the following average values for n: 3.59 for larvae, 3.01 for 0- and 1-year-old fish, 3.20 for immature females of 2 years or more, 3.24 for immature males of 2 years or more, 3.28 for mature males, and 3.40 for mature females. Most of these values differ significantly from 3.0.

The constant, a, can be used to compare an individual with others of the same species. The specific gravity of fish flesh does not vary much within a species so that the value of a will depend on the fatness, being high in fat fishes and low in thin fishes. Since a can only be calculated after n is established, fatness is usually estimated as the "condition factor" or "ponderal index," which is the ratio between the observed weight and the length cubed. Thus, the condition factor, $c = W/L^3$, and its value will depend on the units used for W and L. For salmonid fish, the usual formula is:

$$K = \frac{100 \cdot W}{L^3}$$

for when W is expressed in grams and L in centimeters, K, the condition factor, is approximately 1.0. Since these fish show little change in length–weight relationship with growth (i.e. $n = 3$), K is a valid index for comparing the fatness of salmonid fish of different lengths.

Where n varies with length, the condition factors K and c must also change with increase in length; for such fishes, it is more useful to eliminate the effects of length and correlated factors by using a "relative condition factor," K_n. In practice the length–weight relationship must be calculated over a wide range so that smoothed mean weights, $\hat{W}$, can be computed for each length group or read off an accurate graph. The relative condition factor is then:

$$K_n = W/\hat{W}.$$

Le Cren found that perch in Windermere show changes in relative condition factor during the year but that there is considerable individual variation.

The condition factor depends on the amount of food in the gut (Le Cren found that this varied between 0 and 2.1% of the body weight in wild perch) and under experimental conditions, fish should be weighed and measured after a constant interval from feeding time. The state of the gonads affects fatness, and the condition factor decreases when mature fish spawn. Le Cren found that nearly all the differences between the seasonal values of condition factor, K_n, for mature and immature perch were due to cyclical changes in gonad weight. After

due allowance has been made for the states of the guts and gonads, differences in condition factor reflect differences in nutritive level or the effects of physico-chemical factors on the fishes.

D. Changes in Shape During Growth

Some fishes, e.g., *Petromyzon, Anguilla, Pleuronectes,* have a metamorphic life history during which the body form is dramatically transformed at least once. In the majority of fishes, however, changes in shape are slight after the yolk sac has been absorbed; these changes are indicated by changes in the exponent, n, of the weight–length relationship, as already described (p. 370).

Many fishes develop secondary sexual characters and some of these involve changes in shape, e.g. the gonopodia of viviparous fishes and the "kype" or lengthened lower jaw of male salmonid fishes; these are attained by differential growth of parts in relation to the length of the body. Such features as the cusp patterns of teeth, often used in taxonomy, may change during life.

On the whole, however, the relative growth of parts of the body may show remarkable isogony with increase in length. This is of great importance when seasonal changes in the structure of parts are used in computing growth histories, as are the "rings" on scales and bones. We shall not, at this time, consider further the shapes of fishes or their parts.

III. THE SIZE HIERARCHY EFFECT

When groups of fishes of one species are kept together, it commonly happens that the dispersion in size between the largest and smallest individual increases as they grow larger. This phenomenon is most clearly seen when all the individuals are approximately the same size at the beginning of the experiment for after a few weeks some may be markedly larger than others (see Fig. 5, p. 384).

It is a normal procedure in trout hatcheries to "grade" the fry at intervals. After a few weeks of feeding, they are separated into groups of large, medium, and small individuals. When these groups are regraded after a few weeks, some of the "small" fish will have become as big as the "large" fish. Brown (1946a) sorted 2 groups of 8-week-old trout fry, *Salmo trutta,* into equal numbers of "large" and "small" fish and grew these under identical controlled conditions giving them as much food as they would eat every day. After 28 weeks, the range of size in the 2 groups was almost the same and the largest "small" fish weighed 8.15 g. and was nearly as large as the largest "large" fish (8.39 g.) and considerably larger than the smallest of the "large" group (0.77 g.). These results are illustrated in Fig. 1. Thus, the smaller fish grow more

slowly than the larger fish because the latter are present but, if the larger fish are removed, the smaller fish are able to grow more rapidly. When trout alevins are ready to feed, some start to swallow food particles earlier than the others and these individuals probably acquire an initial advantage in size which they can then maintain. The fish in these experiments were given more food than they would eat and there was no evidence that the growth of the smaller fish was restricted by shortage of food.

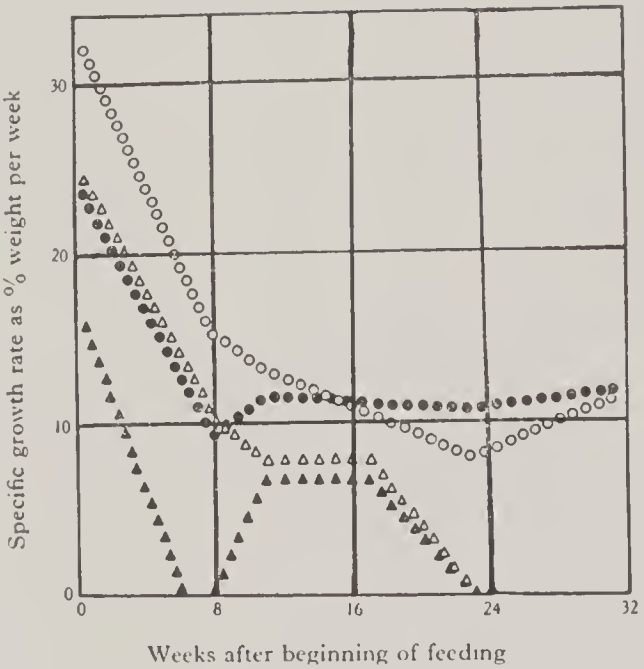

FIG. 1. The specific growth rates of four individual trout fry, all grown in the same environmental conditions. ○ This fish was the largest individual in its group throughout the experiment. Before week 8, the group consisted of large and small fry; after week 8, of large fry only. △ This fish was the twentieth in the order of decreasing weight for its group during the first 8 weeks. It then became the smallest individual in a group consisting of large fry only. ● This fish was the twenty-first in the order of decreasing weight for its group during the first 8 weeks. It then became the largest individual in a group consisting of small fry only. ▲ This fish was the smallest individual in its group throughout the experiment. Before week 8, the group consisted of large and small fry; after week 8, of small fry only (from Brown, 1946a).

Trout fry show territorial behavior at an early age and can be recognized clearly as individuals. Stringer and Hoar (1955) observed a "peck order" (order of dominance) among young *Salmo gairdnerii kamloops* and refer to similar behavior in *Oncorhynchus kisutch;* the larger fry are more aggressive than smaller ones and, when the largest individual is removed, another large individual becomes the dominant fish. The size hierarchy effect on growth is probably related to this order of dominance with the largest, dominant fish growing fastest. Similar orders have been

reported for other speeies of fishes and the size hierarehy effect also oecurs among fish other than salmonids, e.g. the herbivorous *Tilapia zillii*. In larger groups of *Salmo trutta* fry, the growth rate of an individual seems to be determined by the number of larger fry present, while in smaller groups, individuals are influenced by the relative numbers of larger and smaller individuals (Fig. 2).

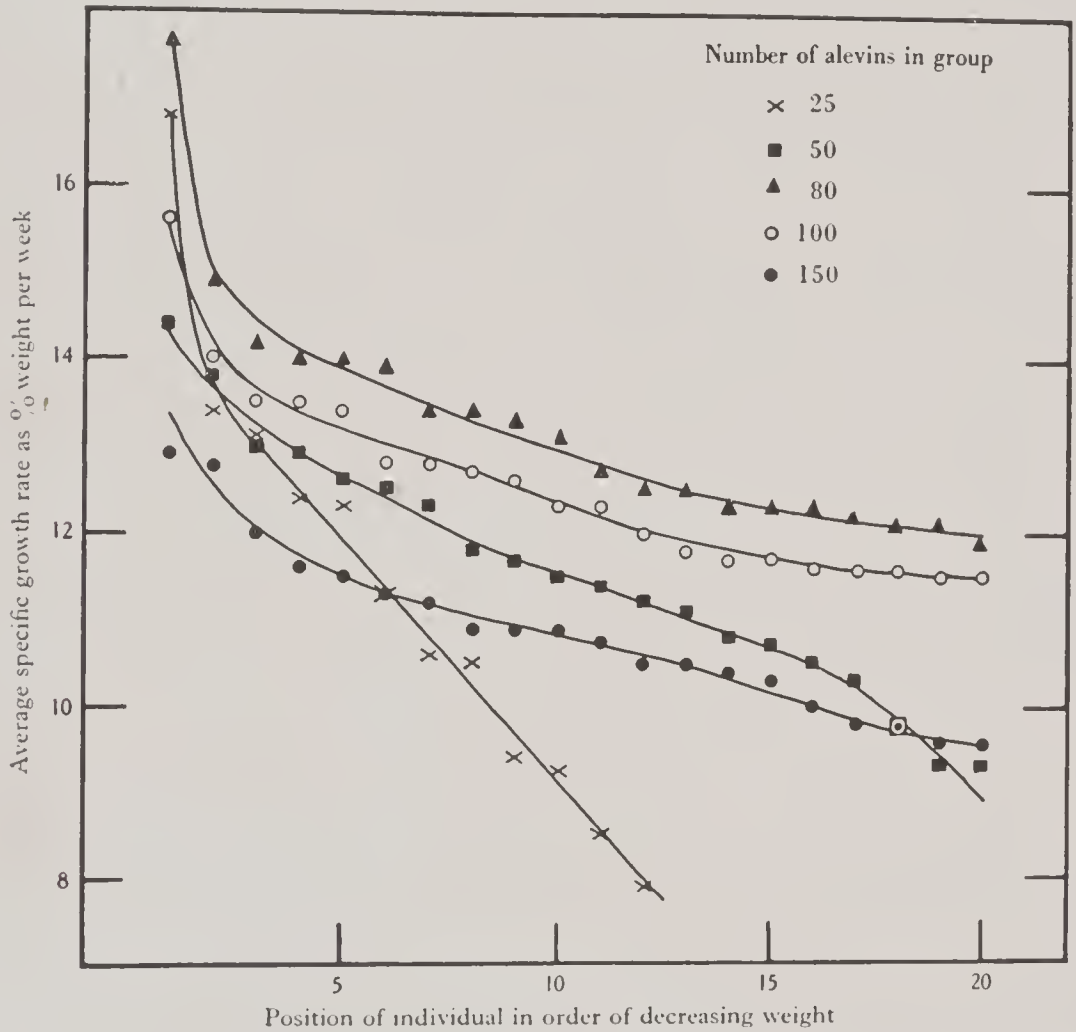

Fig. 2. Mean specific growth rates for 20 weeks after the beginning of feeding of the 20 largest individuals in groups containing different numbers of individuals (from Brown, 1946a).

While the size hierarehy effeet ean be eompared with the "peek order," its mode of operation requires investigation. The order of dominance in salmonids depends on visual stimuli; trout generally feed by sight. When food is added to a tank with trout of different sizes, they all swim towards it and there is no obvious reluctance of smaller fish to feed in the presenee of larger ones. When some food remains on the bottom of the tank, the small fish have ample opportunity to feed after larger ones have satisfied their appetites yet, even when the food consists of live and moving organisms, the small fish do not grow as large as the larger

ones. It is possible that they are inhibited from feeding satisfactorily by the presence of the larger individuals. Another possibility is that the smaller fish suffer from "stress" in the presence of larger ones and that their production of adrenocorticotropin (ACTH) is increased. In *Astyanax*, such an increase is associated with marked decrease in growth (Rasquin and Rosenbloom, 1954, and see Chap. VI); the "stress" was caused by continuous darkness.

The size hierarchy effect is always very marked among groups of brown trout and in a series of experiments the dispersion in size between the largest and smallest members of each group may be such that the values for the different groups show considerable overlap. There are various possible ways of comparing these groups. When histograms showing the length or weight frequency distributions are compared, it is often clear either that the majority of fishes in certain groups have done better than those in others (see Fig. 5, p. 384) or that the frequency distributions are very similar. If the groups contain different numbers of individuals, it is possible to compare either the mean sizes and their standard deviations or the median values and interquartile ranges. The mean value for a group is considerably influenced by the actual sizes of the largest and smallest fishes, particularly by the former when the dispersion is very great. The actual size of the largest fish is a matter of chance for it depends on how effectively this fish establishes its dominance at an early stage of the experiments; in replicate groups, values for the largest fish may differ considerably. It is therefore probably more useful to compare median values and their derivatives rather than mean values if the groups consist of more than about twenty individuals.

One of the drawbacks of experimental work on the growth of individual fishes is the difficulty of collecting sufficiently numerous records unless the available facilities include a very large number of tanks and plenty of assistants. To a professional statistician, the available data must often appear meager and comparison of results by refined statistical methods is therefore unprofitable. However, effects of biological and economic importance should cause differences big enough to be obvious to inspection. Where differences can be established only by statistical treatment, the effects are probably of little significance in natural conditions. Since the number of fishes used in experiments is often not very large, it is of very great importance that the conditions be clearly described and as fully controlled as possible so that experiments may be repeated if necessary.

Where considerable numbers of fishes are used, e.g. in trout hatcheries, all the fishes cannot be recognized as individuals and sometimes only limited samples are caught for measurement. Here all the problems

associated with sampling errors may be involved. Another problem results from the death of some of the individuals during the experiment. When trout alevins begin to feed, some do so early but others never feed successfully and these die during the first few weeks. Among older trout, sudden adverse conditions are more likely to be fatal to the larger fish if they are all in good condition. Average sizes recorded before and after periods of high mortality may differ very much if the dead fish were above or below the average size and some allowance must be made for this.

IV. EFFECTS OF PARENTAL FACTORS ON GROWTH

Among mammals, e.g. horses and rabbits, adult size depends on genetic constitution (inherited from both parents) and on embryonic environment (depending on the female parent), as well as on nutritive and other factors acting during the juvenile period. "Races" of fishes of the same species are generally recognized by differences in behavior or in meristic characters, such as numbers of vertebrae, which may depend on environmental factors. There have been few investigations of the extent to which characteristic differences in growth patterns may result from parental factors. Experimental data are available only for "trout" and even these are not very extensive.

A. Egg Size

Many fishes have yolky eggs and the embryonic periods may be compared in some ways with the fetal life of mammals. The size of the eggs, and hence the amount of yolk available as food for the developing fish, depends on the female parent (see Chap. VII). The number of eggs produced at each spawning is determined early in the reproductive cycle; in general, larger fish produce more eggs than smaller fish of the same species from the same water. The final size of the eggs depends both on the size of the parent and on her level of nutrition during the period preceding spawning; larger fish and fish with a more abundant food supply generally produce larger eggs. Thus, as a female fish grows larger, she will produce a greater number of larger eggs unless there are changes in her environment.

Embryonic nutrition is discussed in Chap. VIII. At the end of the embryonic period, when the young fishes are ready to begin to feed, those derived from larger eggs are slightly larger than those derived from smaller eggs if they have been reared under identical conditions. If it is true that the fish from the larger eggs grow faster than those from smaller eggs and thus increase the initial difference in size, the use of larger fish as parents should become the rule in commercial fisheries.

The evidence is conflicting for most of the few experiments have been insufficiently controlled. On balance, the larger alevins probably maintain a greater size for some months because their specific growth rates are the same as those of the smaller alevins. However, the size hierarchy effect has so marked an influence on individual sizes that the weight frequencies may overlap almost completely (Brown, 1946a). Alm (1939) compared fry from small eggs (from river trout) and from large eggs (from lake trout) under hatchery conditions. The alevin lengths were 23–24 mm. and 27 mm., respectively, and after 1 year, the ranges in lengths were 4.8–7.3 cm. and 5.5–8.1 cm., respectively. Alm found that the 2 sets of fry did almost equally well under crowded conditions but the larger alevins grew slightly better under conditions of very little crowding.

B. Genetic Factors

Higgins (1929) and Davis (1934) successfully selected breeding stock of *Salvelinus fontinalis* for rapid growth. Davis' fish attained in one year the size normally reached in three or four years but his results are complicated by the fact that they were also given "improved" diets.

Millenbach (1950) reported that selection of *Salmo gairdnerii* for early maturation was accompanied by so marked a retardation of growth that the experiments were discontinued. This suggests that age of maturity and rate of growth are both influenced by genetic factors in this species.

Alm (1939, 1949) found that the age of first spawning of *Salmo trutta* is genetically determined. Trout from a small stream became sexually mature when three years old whereas those from a large lake did not mature until about five years old; the wild fish in the lake grew faster than those in the stream. When offspring from these two races of trout were reared under identical hatchery conditions, they grew at similar rates for their first three years but the stream fish matured at about three years and the lake fish at about five years old and these differences in reproductive development have persisted through several generations descended from the two races. The young "lake" trout grew slightly larger than the young "river" trout but they were also derived from eggs of different sizes (see above).

Brown (1946a) compared the early growth of *S. trutta* fry which had the same male but different female parents, though all were of the same age. The two batches of eggs had overlapping size distributions so it was possible to compare alevins of approximately the same size. The fry from one family grew faster during the first few weeks but those of the other family showed less rapid decrease in specific growth rate and were

markedly larger at the end of eight months. Dispersion in size, because of the size hierarchy effect, meant that there was considerable overlap in the weight distributions of the two groups. Alevins of different sizes grew at similar rates in one family whereas in the other, the smaller alevins initially grew faster and thus attained nearly the same size in eight months as the larger alevins.

Parental factors thus influence the growth patterns and rates of trout. In species of fish which lay eggs with very little yolk, the factor of egg size can be of little importance for early growth will depend on the external food supply. Even for trout, the effect of egg size is probably negligible in comparison with other factors. Genetical factors are difficult to assess but since they may control the age at which fish mature, they may affect considerably the maximum size likely to be attained by different races as well as affecting the growth patterns of immature fishes to some extent. Races of fishes, such as herrings, *Clupea,* which differ in time and place of spawning may differ also in the growth they could make under identical conditions.

V. GROWTH CYCLES AND CHANGES IN GROWTH RATE WITH AGE

It might be expected that fish living in a constant environment with an unrestricted food supply of constant quality would grow at a constant or constantly decreasing specific growth rate but this may not occur in practice. Brown (1946b) used constant temperature (11.5° C.), 12 hours per day of artificial light, constant rate of water flow and fed two-year-old trout, *Salmo trutta,* on liver, allowing them to eat as much as they would at regular feeding times. There were differences between individual specific growth rates, depending on size hierachy, but averaged monthly growth rates showed the same trends for all the fish. They were 88 weeks old at the beginning of the observations and their growth rates decreased to a minimum in October-November, rose to a maximum in February, fell gradually throughout the summer until August, and then decreased markedly. At this stage, the fish became very excitable and several died and were found to be nearly sexually mature. Thus, these fish were ripening as they approached the end of their third year of life; the surviving fish were stripped of eggs and milt in November and their specific growth rates began to increase again in January. These fish had lived in a constant environment for more than a year and yet maintained a physiological cycle which led to maturation of the gonads at the same time of year as under natural conditions. This physiological cycle was associated with changes in specific growth rate, with maximum rates from February to May and minimum rates in October and November.

The existence of this growth rate cycle under constant environmental conditions means that fish in experiments must be compared with "control" fish of the same age since fish of different ages will be at different stages of the growth cycle. Swift (1955) found a reproducible growth cycle in three-year-old trout under hatchery conditions. Temperature and light varied through the year; the spring increase in growth rate did not begin until March, it was maximum in May; the autumn decrease in rate occurred in October and growth rates were high in August and September; there was a marked minimum in June-July. Swift suggests that this minimum was correlated with illumination (see below); the other differences from Brown's annual cycle can be correlated with differences in temperature (see below).

Besides the annual growth rate cycle, Brown (1946b) found that most of the two-year-old trout showed an alternation of periods of rapid and slow growth in weight of about two weeks (at 11.5° C.). The specific growth rate for length was correlated directly with the specific growth for weight during the preceding two weeks so that fluctuations in these rates alternated with each other. Swift (1955) noted that his monthly averages for growth rates for weight and length varied always in the same way but the cycle for weight preceded that for length.

The specific growth rate for length of Brown's fish was correlated directly with the condition factor, K (see p. 371), good condition being associated with rapid growth. The relation between the specific growth rates for length and weight at 11.5° C. is such that the greater is the divergence of K from 1.08, the greater is the change in its value after 2 weeks towards this value. Trout with K less than 1.08 grow relatively faster in weight than in length while fish with K more than 1.08 grow relatively faster in length.

When fishes spend their whole lives in one environment, their growth rates generally decline progressively as they grow older (after allowance for annual cycle, etc.) but a change in environment (including food supply as part of the environment) may be associated with a spectacular change in growth rate. Thus, salmon grow considerably faster in the sea than during their last year in fresh water and wild populations of trout, *Salmo trutta*, display the same phenomenon if they move from small moorland streams into lakes. There is some evidence that the capacity to show increased growth under better environmental conditions declines with age. Comfort (1956) figures growth curves for individual female guppies, *Lebistes*, in containers of different sizes and on different feeding schedules; these fish responded to more food and/or space by increased growth even when they were 500 days old but the response of these older fish was less marked than that of fish only 350 days old.

Comfort states that he has kept female guppies under conditions such that they remained 2 cm. long for 600 days; they were sexually mature but still capable of resuming growth in improved conditions. Frost and Smyly (1952) found slight increases in growth rate even in 4-year-old trout after addition of bone meal to a tarn but increases were much more marked in fish of 1 or 2 years old.

VI. THE RELATION BETWEEN FOOD AND GROWTH

Food supply is probably the most potent factor affecting the growth of fishes, for only if the food supply is sufficient in quantity and adequate in quality can fishes attain the maximum growth possible for the existing physico-chemical conditions. The effect of some of these, e.g. temperature, on rates of growth is also largely the result of effects on feeding and on food requirements.

It is not easy to measure accurately the food intake of fishes. For those which live on microscopic food, the problem is probably insuperable and the best that can be achieved is to provide "unrestricted" or "restricted" supplies. If the fishes feed on particles of reasonable size, the situation is easier; it is possible to add a weighed amount of food and to recover, with a siphon, any food particles remaining when the fish have finished feeding. Feces may or may not be removed with the recovered food. There are, however, two difficulties: (1) the fish may break up the food as they eat it and eject very small particles which cannot be recovered; (2) the recovered food remains may have absorbed water so that the weight eaten cannot be obtained simply by subtracting the weight of food recovered from the weight of food given. Unless the food consists of live animals which can be captured and weighed intact, samples should be used for estimation of dry weights and the food remains, with or without feces, should also be dried; the dry weight of food absorbed by the fish can then be estimated by subtraction and converted back into wet weight if desired. The estimate will probably be higher than the true food eaten, because of losses of food and food remains by solution, but the method should give consistent and repeatable results if there is a definite feeding and cleaning schedule.

In large scale experiments, the problem of obtaining adequate supplies of live organisms, such as *Gammarus*, is likely to be prohibitive so that it is necessary to use unnatural foods such as beef liver, meat, lettuce, or mixtures of meals with added vitamins and salts. These are easier to obtain and handle in bulk and their use ensures that the quality of the food remains constant during the course of the experiment.

Another possible complication arises from the suggestion that some fishes, e.g. carp, *Cyprinus carpio*, can absorb substances in solution

through their skins. If these fish are supplied with water which has passed through a tank containing other fish, they may obtain food in solution in addition to that intended for them.

A. Maintenance Requirements

Some of the food absorbed must be used to replace tissues broken down in catabolic processes, i.e. to provide for "basal metabolism." Basal metabolic rates can be measured by studying the respiration of anesthetized fish (see Chap. I: Part I.). Normal fish spend part of their lives resting and part in activity and the degree of activity is characteristic of the environmental conditions, being influenced especially by temperature. The energy for this activity must be obtained from the food. Fish can gain weight only if they eat more food than is necessary to satisfy their basal metabolism and to provide energy for their activity (i.e. to balance their "routine metabolism"). The amount of food which a fish must eat if it is neither to gain nor to lose weight is the "maintenance requirement" or ration and this provides for the routine metabolism. It will vary with environmental factors and may vary with the size, age, and physiological state of the fishes.

Brown (1946b) measured the maintenance requirements of groups of two-year-old trout, *Salmo trutta*, under standard conditions. The fish were weighed and measured weekly and the change in weight was plotted against the amount of food absorbed. As the amount of food added was reduced, the fish first lost weight and then, becoming "adapted" to the new level of feeding, gained weight so it was necessary to continue reducing the amount of food given until there was no further adaptation. The change in weight was then directly proportional to the amount of food absorbed, and the maintenance requirement was read from a graph showing at least three consecutive observations (see Fig. 3). When the amount of food given was increased again, the fish soon became "adapted" to the higher level of feeding and might lose weight in one week when the amount of food absorbed was greater than the maintenance requirement. Thus, the fish showed maximum efficiency of utilization (see p. 386) of the food at the maintenance level, with net conversion factors of about 100%. Although the fishes in the group were of different sizes, they all gained and lost weight in the same weeks and seemed to obtain proportional shares of the restricted food supply.

Under standard conditions at 11.5° C., the maintenance requirement decreased relatively as the fish grew larger, reaching a constant level of about 60 mg. meat (of 22.5% dry weight) per gram fish per week for trout weighing 100 g. or more. Three-year-old trout in November gave the unexpectedly low value of 28 mg. per gram per week possibly

because their well-developed gonads could be resorbed and used very efficiently for maintenance.

Brown (1946c) measured the maintenance requirements of trout under the same standard conditions but at different temperatures. The relationship between requirement and temperature was not simple; for fish

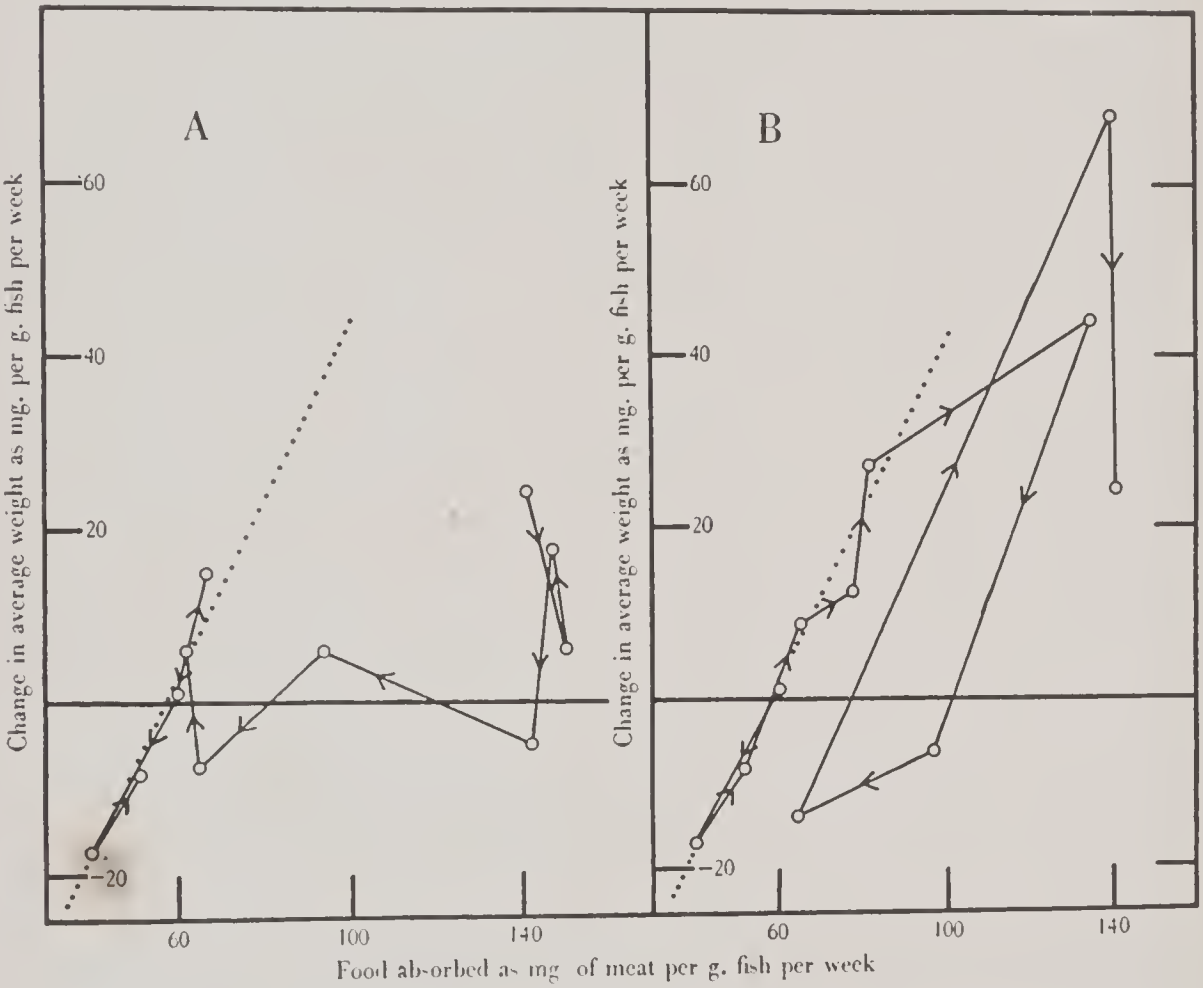

Fig. 3. Adaptation of trout to different levels of feeding. A: the amount of food added was reduced towards the maintenance level. B: the amount of food added was increased above the maintenance level. The dotted line represents the relation between the amount of food absorbed and the change in average weight at the maintenance level. Values for consecutive weeks are joined by straight lines, and the arrows show the order in which the values were obtained (from Brown, 1946b).

of equal sizes, maintenance requirements increased gradually from about 5° C. to about 9° C., then rapidly to about 11° C. and less rapidly above this, becoming nearly constant at about 20° C. (Fig. 4). Brown interpreted this relationship as the result of variation of activity with temperature. She suggested that the trout were most active between 10 and 12° C. and that the reduction of activity at higher temperatures (17–20° C.) was sufficient to compensate for the expected increase in basal metabolism. The maintenance requirement should be directly

related to "routine metabolism" and the data for *Salvelinus fontinalis*, shown by Fry as Fig. 23 of Chap. I: Part I (p. 48) agree remarkably well with the data for *Salmo trutta* quoted above. At temperatures above 20° C., individual trout would probably become much more active as the lethal temperature is approached, with corresponding increase in maintenance requirements.

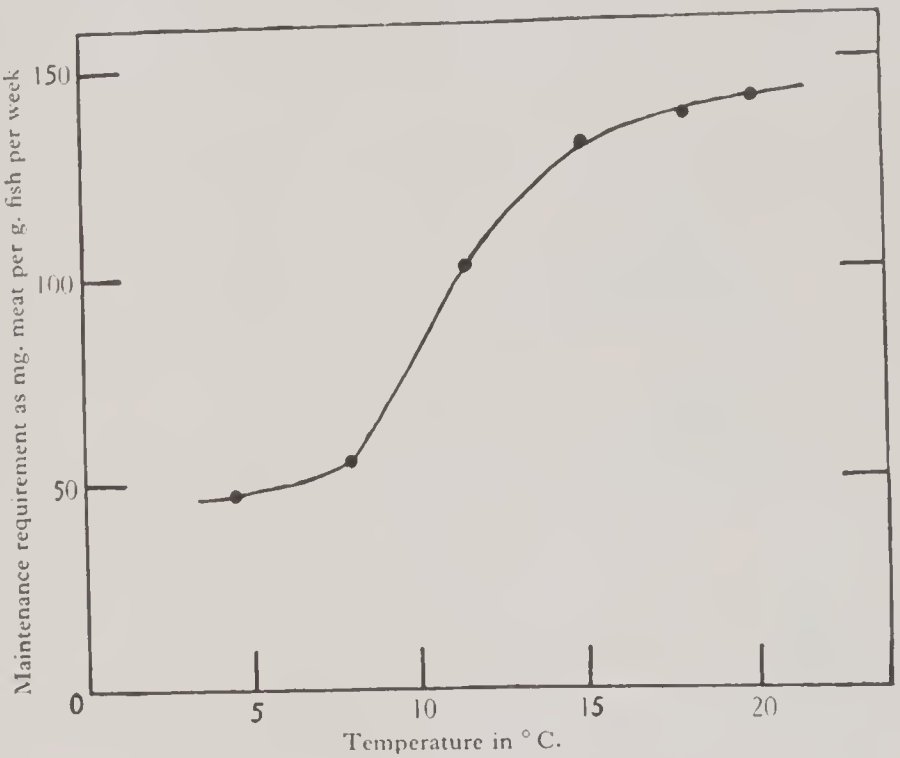

FIG. 4. To show the relation between the maintenance requirements calculated for fish of average weight 50 g. and temperature (from Brown, 1946c).

Pentelow (1939) measured the maintenance requirements of *Salmo trutta* of about 20 g. weight at varying temperatures, mostly between 7 and 10° C. Most of his values were between 70 and 102 mg. *Gammarus* per gram fish per week; this agrees well with Brown's figure of 55 mg. meat per gram fish per week for a 50-g. trout at 8° C.

Dawes (1930) measured the food intake of plaice, *Pleuronectes*, kept separately in live boxes in the sea, and fed with fragments of *Mytilus* flesh. The maintenance requirements of plaice of about 40 g. weight varied between 110 and 240 mg. *Mytilus* per gram fish per week and increased with temperature and decreased with increasing size of individual over the range investigated.

Fish typically lose weight if given no food; the loss of weight is more rapid at higher temperatures (Pentelow, 1939). The fishes can, however, adapt themselves to starvation by lowering their metabolism. Thus, Phillips and Brockway (1954) found that starved brook trout, *Salvelinus*

fontinalis, had a lower metabolic level after 63 hours than after only 15 hours without food. Adelman, Bingham, and Maatch (1955) starved brook trout for seven months at 9.5° C. (over winter). The three batches of 40 fishes were of graded sizes and 70% of the smallest ones (9 cm.) died compared with 10% and 25% of the larger fish (14 cm. and 19 cm. long). During the last 4 months of starvation, the fish lost no weight but there are no figures to show whether they were replacing body proteins, etc., with water. Phillips (1954) analyzed the livers of starved brook

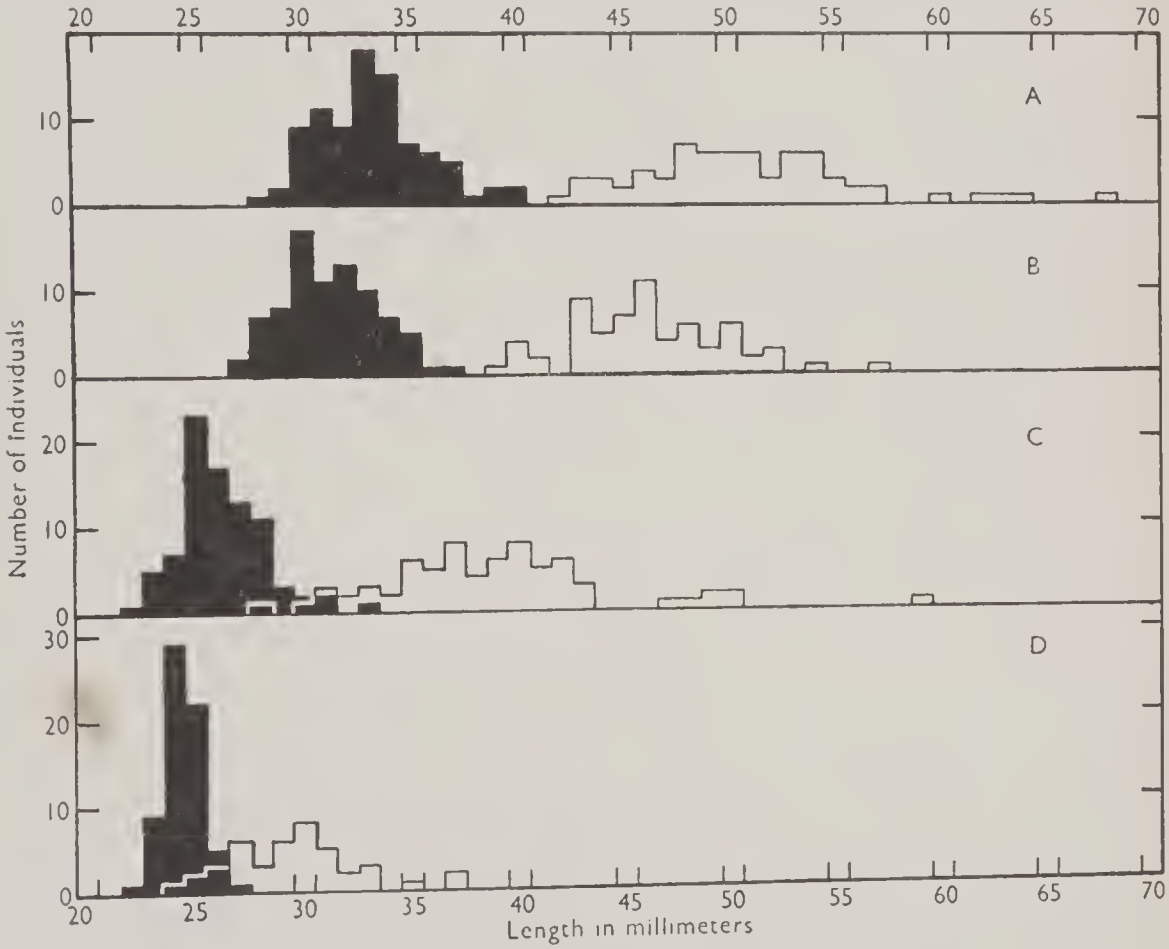

Fig. 5. Histograms to show the distribution of length among groups of trout fry fed with liver at 12.5° C. Solid black: distribution after 5 weeks; white: distribution after 11 weeks. A, fry fed 13 times per week; B, fry fed 7 times per week; C, fry fed 3 times per week for the first 4 weeks and 4 times per week thereafter; D, fry fed once per week for the first 4 weeks and twice per week thereafter (from Brown, 1951).

trout at 9.7 and 10.1° C. These had decreased in weight from 10.3 to 6.2 g. during the 14-week experiment and their livers showed reduction of riboflavin, biotin, and pantothenic acid but increase of niacin; the level of glucose in their blood was low.

Adelman *et al.* (1955) fed their surviving brook trout at different levels

after seven months starvation; those allowed to eat as much as they would, died, but those fed on restricted rations which were gradually increased, survived and resumed normal growth.

B. Appetite

Moore (1941) measured the amount of food eaten by *Lepomis cyanellus, Helioperca incisor,* and *Perca flavescens;* even with an unlimited food supply, only a limited amount was eaten daily. Brown observed that individual *Salmo trutta,* ranging in age from alevins just ready to feed to trout more than 3 years old, have definite appetites and will not eat more even when plenty is available. Brown (1951) compared young trout fry fed 2, 4, 7, and 13 times per week and allowed to eat as much as they would on each occasion. The fry fed only 2 or 4 times per week ate so much on each occasion that they bulged visibly for several hours afterwards, whereas fry fed once or twice daily showed less enthusiasm and never ate sufficient food to distort their streamlined shapes. The fish soon learnt to expect their food at regular times and the amount of growth varied with the frequency of feeding (Fig. 5).

The amount of food eaten thus depends on the previous regime of the fish; it also depends on environmental factors, such as temperature and crowding. Two-year-old trout with unrestricted food supply have appetites which vary from day to day and even from week to week (Brown, 1946b, c). It is probable that cycles of variation in appetite accompany the short-period cycles of growth in weight and length (see p. 379); at 11.5° C., appetite was greatest for trout with K (condition factor) of 1.12 and fell off for values of K above and below this. The amount eaten per gram of fish decreased as the fish grew older and larger; trout with well-developed gonads had a relatively small appetite.

Similar variations in appetite were observed by Pentelow (1939) for yearling trout and Dawes (1930) for plaice.

The effect of temperature on the appetite of 2-year-old trout is such that it is maximum between 10 and 19° C. and falls off above and below this temperature range (Brown, 1946c). The effect of crowding at 11.5° C. is such that very crowded fish (with 3 liters space per fish) eat less and show less fluctuation in appetite; fish with much living space (50 liters per fish) have very considerable fluctuations in appetite (Brown, 1946b). Fish with intermediate amounts of living space have the largest appetites.

Fish given more food than their maintenance requirements but not allowed unlimited food have appetites which fluctuate but they generally eat amounts which vary directly with the amount of food provided.

C. The Efficiency of Conversion of Food into Fish

When calculating the productivity of natural waters or the economics of production in a trout hatchery, the efficiency of conversion of food into fish is an important consideration. The following values have been quoted for brown trout, *Salmo trutta:*

Diet	Pounds Food Required to Give 1 Pound Fish
"Natural foods" in hatcheries	2.3–7.1
Surber (1935) using *Gammarus*	6.6
Pentelow (1939) using *Gammarus*	5.0
Schaeperclaus (1933) using *Gammarus*	3.9
Schaeperclaus (1933) using chironomids	4.4
Cornelius (1933) using chironomids	2.3
Cortland hatchery, using housefly maggots	7.1
Cortland hatchery, using special diet	3.0

The considerable differences between some of these figures are explained, at least in part, by differences in the size of trout observed and in environmental factors such as temperature. Another important consideration, scarcely ever recognized, is the rate at which the fish are growing, for part of the food is used for maintenance and this part will be greater, the slower the growth rate. In fact, there are two possible ways of calculating the efficiency of conversion and only one of these takes account of maintenance requirements.

The figures listed above are all calculations of "gross efficiency" since they represent the change in observed weight of the fish divided by the total weight of food eaten (or provided). The "net efficiency" can be obtained by subtracting from the weight of food eaten the weight of food required for maintenance, so obtaining the weight of "food available for growth" and then dividing the weight increase of the fish by this amount. The following figures show how "gross efficiency" must depend on the growth rate if "net efficiency" remains constant:

A brown trout weighing 50 g. has a maintenance requirement at 11.5° C. of 102 mg. per gram fish per week = 5 g. meat per week.

The net efficiency of conversion at this temperature is 22% so that for each increase in weight of 1 g., the trout must eat 4.5 g. meat more than its maintenance requirement. Therefore:

Total Food Eaten in Week (g.)	Maintenance Requirement (g.)	Increase in Weight of Fish (g.)	Gross Efficiency	
			Grams Food per Gram Increase	Per Cent
9.5	5	1	9.5	10.5
14	5	2	7	14.3
23	5	4	5.75	17.5
32	5	6	5.3	18.7

The effects of age and size and of environmental factors on net efficiency have not been investigated to any extent. Brown (1946b, c) found that the net efficiency varied from week to week for 2-year-old trout at 11.5° C. with unrestricted food supply and was greatest for trout with condition factor, *K*, of about 1.12; larger changes in weight were correlated with higher efficiencies. When the food supply is restricted, the net efficiency is greater and it is consistently very high at the maintenance level (see p. 381). With more food than the maintenance requirement but less than unlimited food, the short-period growth cycles are exaggerated and very high values for net efficiency (more than 100%!) may be recorded during the phase of growth in weight. Trout on restricted rations grow, on average, almost as fast as those on unrestricted rations in the same conditions. Dawes (1930) made similar observations on plaice; fish allowed to eat twice as much as their maintenance requirement per week attained almost the same sizes as fish allowed to eat as much as they would. The latter had a net efficiency of 4–5 g. *Mytilus* for 1 g. increase in weight.

The net efficiency of 2-year-old trout varied with temperature and was maximum at about 8° C. and above 17° C.; it was low between these two temperatures when the fish were most active (p. 382). The effect of crowding at 11.5° C. was to reduce considerably the net efficiency of the very crowded individuals which were also the most active; the other average values were very similar.

The net efficiency of conversion of food may well vary with the type of food used but figures illustrating this are difficult to find. Surber (1935) gives the growth rate of his trout so his figure for gross efficiency of 6.63 g. *Gammarus* per gram increase can be compared with 6.3 g. meat per gram increase, calculated from Brown's data for trout of the same size, growing at the same rate at the same temperature. Pentelow's (1939) figure for net efficiency of 3 g. *Gammarus* per gram increase can be compared with 3.6 g. meat per gram increase for larger fish (50 g. compared with 20 g.) at this temperature.

D. The Quality of the Food

Few fishes restrict their diet to a single species of organism or even to a small number of species. They can usually be described as carnivorous or herbivorous but some, e.g. roach, *Rutilus rutilus*, are omnivorous. Generally the carnivorous can be subdivided into piscivores, insectivores (which include in their diet invertebrates other than insects), and zooplankton feeders; the herbivores can be divided into phytoplankton feeders and feeders on aquatic angiosperms.

The nature of the available food may be especially important at certain stages in the fish's life for many fish change their habitats as they

grow larger and many change their diet. The larger perch, *Perca fluviatilis*, and trout, *Salmo trutta*, in lakes such as Windermere are those fish which have adopted a piscivorous habit and individuals of comparable age and size are not found in waters where there are no suitable fishes to act as food. When fish move into waters with more abundant food, changes in growth rate are registered on the scales.

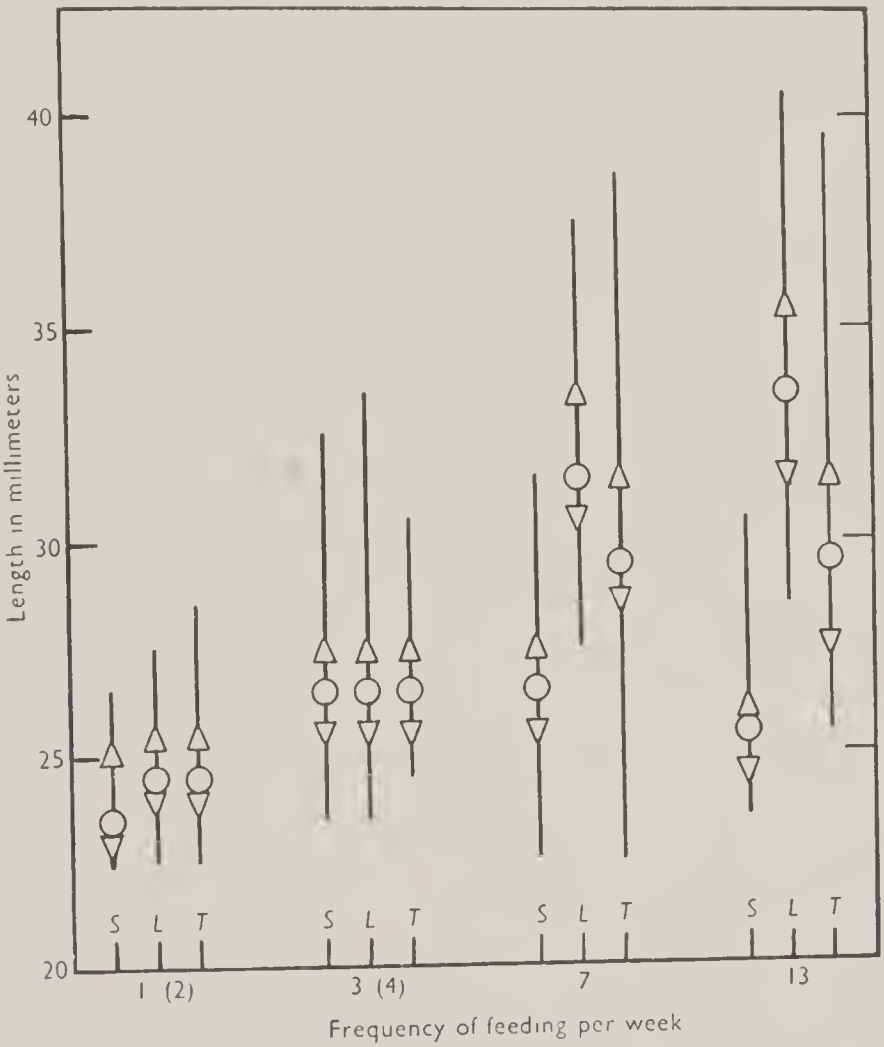

FIG. 6. The distribution of length among groups of trout fry at 12.5° C. after 5 weeks of different diets. The heights of the vertical lines equal the total ranges in length. ○ median value; △ upper quartile; ▽ lower quartile; S, diet of shrimp meal; L, diet of liver; T, diet of *Tubifex* (from Brown, 1951).

Trout are relatively unselective carnivores and it might be expected that any animals or parts of animals would be equally suitable as food. Myers (1946), however, has suggested that Cladocera and other zooplankton are the ideal food for trout fry and far superior to meat and liver. Brown (1951) grew trout fry under identical conditions for five months on three different diets. Shrimp meal reinforced with occasional feeds of live *Daphnia* suspension was not at all satisfactory; the fry grew

well on beef liver, while on live *Tubifex*, they grew well but developed pathological symptoms as they reached a certain size. Groups of fry were given different amounts of these diets (Fig. 6) and growth was very similar when the fish were fed up to four times a week. Fry fed daily with liver were, however, markedly larger after 5 weeks than those fed daily with *Tubifex* and the difference was even more marked between the groups fed twice daily. Thus, the quality of the food affected the growth achieved only when there was plenty of food; when the amount was restricted, quality had no influence on the rate of growth.

E. Dietary Requirements

The feeding habits of fishes show great variety, as already indicated, and the many types of food organism must differ in the relative proportions of proteins, carbohydrates, etc., and also in the actual identity of the proteins, vitamins, etc. The dietary requirements of different species are probably quite different but only those of salmonid fishes have been investigated in any detail.

Much of the critical work on "trout" (*Salvelinus fontinalis, Salmo gairdnerii,* and *S. trutta*) has come from the Cortland Hatchery, New York, and this is recorded in the Annual Reports of that organization (see Phillips *et al.*, 1954, for details). Various artificial diets have been tested at the Cortland Hatchery and recommended feeding schedules and diet sheets have been issued to trout propagators (e.g. Deuel *et al.*, 1952). Cortland diet number 6, given below, is an example of a satisfactory artificial diet:

Composition of Diet	Percentage by Weight in Cortland Diet No. 6
Beef liver	15
Beef melts	35
Wheat middlings	12
Cottonseed meal	12
Whitefish meal	12
Dried skim milk	12
Salt	2

The substitution of an equivalent weight of dried distillers' solubles for the dried skim milk reduces the cost of this diet (in the United States) and yields an increase of 13% in the weight of trout produced per pound of food (Corson, 1954).

Earlier workers postulated that a satisfactory artificial diet should be similar in the proportion of its constituents to the natural diet of the fishes. However (Phillips, Nielsen, and Brockway, 1954), the successful

Cortland diet No. 6 differs very considerably in chemical composition from averaged values for organisms characteristic of a trout's natural diet (see Tables II and III). Most of the values for the artificial diet lie outside the very considerable range in values among the food organisms analyzed.

TABLE II

PERCENTAGES BY WEIGHT OF CERTAIN CONSTITUENTS IN DIETS OF TROUT [a]

Diet	Water	Protein	Fat	Ash	Calcium	Phosphorus	Magnesium
Natural foods [b]							
average	82.2	11.5	2.2	1.7	0.100	0.153	0.022
minimum	72.5	6.4	0.7	0.8	0.023	0.114	0.010
maximum	87.5	17.9	6.1	2.7	0.266	0.220	0.033
Cortland diet No. 6	41.4	28.5	3.2	6.3	0.706	0.806	0.083

[a] From Phillips, Nielsen, and Brockway, 1954.

[b] The natural foods were 5 species of insect larvae, *Gammarus*, and *Helodrilus*.

TABLE III

COMPARISON OF CERTAIN VITAMINS IN DIETS OF TROUT [a]

Diet	Riboflavin (μg./g. food)	Biotin (μg./g. food)	Pantothenic Acid (μg./g. food)	Niacin (μg./g. food)
Natural foods [b]				
average	12.3	0.322	16.3	28.1
minimum	2.0	0.151	10.9	10.3
maximum	33.5	0.437	20.1	38.1
Cortland diet No. 6	13.9	0.520	22.6	42.8

[a] From Phillips, Nielsen, and Brockway, 1954.

[b] The natural foods were 6 species of insect larvae.

The artificial diet would appear to have a higher food value: trout living on it grow at rates comparable with those of fast-growing trout in natural conditions and their gross efficiency of conversion factors are of the same order. Thus, the natural diet must have some advantages—perhaps, as Phillips *et al.* suggest, the amino acids have a greater biological value or there are growth-promoting substances as yet unidentified.

If artificial diets are to be used, it is essential that the fish should eat them readily and, for this, factors other than food value may be important. Thus, if trout are given food pellets, they must be trained to take them; fish which have been reared on meat learn quickly to ingest red pellets but ignore blue, green, tan, and yellow pellets which are identical except for coloring matter (Wolf, 1953).

Experiments on the addition of vitamins to trout diets began more than thirty years ago (Davis and James, 1924) and the vitamin requirements of brook and rainbow trout have been fairly fully established, especially with regard to the B group (McLaren *et al.*, 1947; Churchill, 1952; Cortland Hatchery reports). As in other animals, deficiencies of certain vitamins may be recognized by definite pathological symptoms, e.g. "blue slime disease" of brown trout is cured by adding biotin to the diet (Phillips *et al.*, 1952); deficiencies of other vitamins are shown by lack of vitality, poor growth, and heavy mortality.

Addition of antibiotics to the diet of farm animals has often improved their growth but so far similar experiments with trout have not been encouraging. The growth rates of fish with fortified diets have been similar to those of controls on artificial diets under good conditions. It is possible that other species of fishes might respond better but this seems unlikely since bacteria play less part in digestion in fishes than in ruminants.

The role of minerals in fish diets has not been investigated but calcium ions, at least, may be absorbed directly from the water, probably via the gills (Lovelace and Podoliak, 1952), and it is possible that other ions may also be absorbed thus (see Chap. IV). The mineral content of the food may then be important only where the water supply is deficient.

Trout are carnivorous and it would be interesting to know in the same amount of detail the dietary requirements of fishes of quite different habits. The nearest approach to such investigations has been in the field of "fish farming" where the economics of adding different types of fodder and fertilizers have been described (see p. 396). Such observations do not add much detail to our knowledge of specific dietary requirements.

VII. THE EFFECTS OF ENVIRONMENTAL FACTORS ON GROWTH

A. Temperature

Temperature alters the rates of metabolic processes and may be expected to have a considerable effect on the growth of poikilothermous animals. Increase in temperature should lead to increased maintenance requirements and, since the fish are more active, to increased food intake. The rate of growth will vary with the ability of the fish to digest more food than is required for maintenance. The expected relationship between temperature and growth is that there will be little or no growth below a certain temperature, above this, the growth rate should increase with temperature to a maximum and then decrease, perhaps becoming negative at temperatures approaching the lethal limit. This relationship was found by Audigé (1921) for *Cyprinus*, *Carassius*, and *Scardinius*

with an optimum temperature for rapid growth at 25° C. It also applies to *Tilapia zillii,* which grow very slowly at 20° C., more rapidly at 25° C., and at a maximum rate at 30° C. At 33° C., a temperature close to the upper lethal limit, their growth is almost as rapid as at 30° C. (unpublished data).

For *Salmo trutta,* however, the relationship is different. Brown (1946c) found two optimum temperature ranges for two-year-old trout, 7–9° C. and 16–19° C. Below, between, and above these temperatures the growth rates were lower. This effect of temperature on growth rate can be explained by considering the appetites and maintenance requirements at different temperatures. As already described (pp. 382, 385), appetite is maximum between 10 and 19° C. and maintenance requirements increased rapidly between 9 and 11° C., then less rapidly to become almost constant at about 20° C. Thus, the optimum temperatures for rapid growth are those for which appetite is high and maintenance requirement relatively low while the minimum growth rates at intermediate temperatures occur where the maintenance requirements is unexpectedly high because the fish show maximum activity. At temperatures below 7° C., maintenance requirements are low but so also is appetite, whereas above 19° C., appetite falls off while maintenance requirements remain at the same level as at 17° C.

Swift (1955) found a remarkably similar relationship between growth and temperature in 3-year-old trout, with optimum temperatures for rapid growth between 8–12° C. and 15–16° C. He noted that the fish were most active and difficult to handle between these temperatures. The data of Pentelow (1939) and Wingfield (1940) also fit this relationship between temperature and growth when allowance is made for the annual growth rate cycle.

Brown (1951) found that the effects of small differences in temperature were apparent only when trout fry were well fed. Fry fed up to 4 times a week grew equally well at 10.5° C. and 12.5° C. but fish fed daily or twice daily were significantly larger after 11 weeks at 12.5° C. than at 10.5° C. (Fig. 7). Felin (1951) found differences between the growth of members of the same brood of *Platypoecilus* at temperatures differing only by 2.3° C. The platyfish at 22.1° C. grew and survived better than those at 23.4° C. (over a period of 19 months).

B. Light

The possibility that light may have some effect on rates of growth is seldom considered, yet there is evidence that certain endocrine organs of fishes are affected by the amount of light (see Chap. VI) and it is to be expected that there may also be effects on growth. Under natural

conditions in temperate zones, the cyclical variations of temperature and light are nearly in phase and this makes it difficult to separate their effects. Swift (1955), however, has shown that seasonal variations in activity of the thyroid gland of 3-year-old *Salmo trutta* can be related to the number of hours of daylight, with peak activity at midsummer. This peak in thyroid activity coincides with temperatures between 12

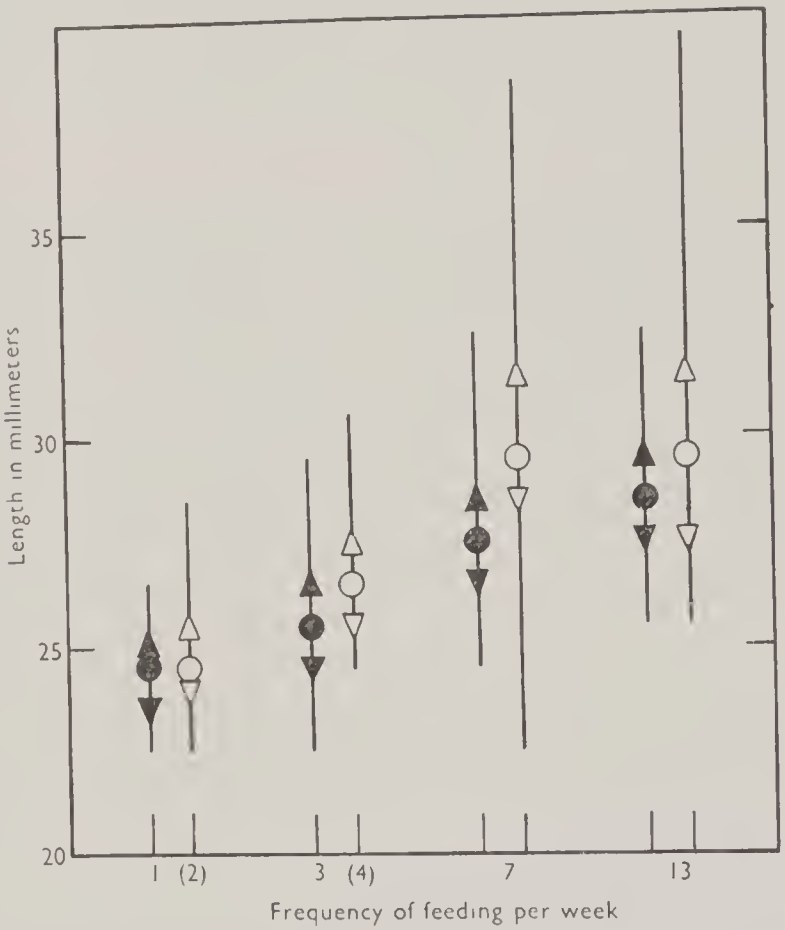

FIG. 7. The distribution of length among groups of trout fry fed for 11 weeks with liver. The heights of the vertical lines equal the total ranges of length. ○ median value; △ upper quartile; ▽ lower quartile; black, fry at 10.5° C.; white, fry at 12.5° C. (from Brown, 1951).

and 15° C. when activity is maximum and growth rate is at a minimum.

Brown (1946b) found that at 11.5° C., under her standard conditions, average specific growth rates were significantly lower with 12 or 18 hours per day of standard light than with only 6 hours of light per day. These fish were fed once a day with more food than they would eat. If feeding entailed search using sight, shorter day-length might be a disadvantage.

Qasim (1955) showed that fry of *Blennius pholis*, provided with abundant food, grew better with 16–17 hours of darkness than in con-

tinuous light; he postulated that these fish required a certain amount of "sleep" for optimum growth.

Fish which normally feed by sight may sometimes live in underground caves in continuous darkness. Under these conditions, brown trout, *Salmo trutta*, can feed and grow successfully; one such trout had fed on *Gammarus* and was in excellent condition and ripe with milt in early December (unpublished data). If, however, individuals of *Astyanax* are kept in total darkness, they develop a condition of "stress" and grow only very slowly (Rasquin and Rosenbloom, 1954; see also Chap. VI).

C. Chemical Factors

Fresh waters differ considerably in their salt content, usually in relation to the soils over which they flow. The most marked differences are in the amounts of calcium, carbonate, and bicarbonate ions (giving waters of different "hardnesses") and the presence and absence of these ions are generally associated with alkaline and acid pH, respectively. Sodium and potassium ions may be present in substantial quantities in some waters and there must be considerable variation in the amounts of other substances, e.g. iodides, in solution. Even in the sea, the composition of the water may vary though not within such wide limits as in fresh waters; in estuaries, there may be considerable changes between low and high tide.

No one has yet demonstrated unequivocally that fish growth rates are directly affected by the ionic composition of the water. There is considerable circumstantial evidence that *Salmo trutta* grows more rapidly in hard, alkaline waters than in soft, acid waters but Brown (unpublished data) found no differences in early growth rates between trout fry grown for 5 months in Cambridge tap water (hard) and those grown in the same water: (1) "softened" so that almost all the calcium ions were replaced by sodium ions; (2) with the calcium and bicarbonate ions reduced to about 5 p.p.m.; (3) acidified with decinormal hydrochloric or nitric acids; and (4) diluted with distilled water so that its salt content was only $\frac{1}{6}$. In none of these experiments, however, was the pH of the water maintained below 7 since the fish produced enough ammonia to neutralize the acid used in (3). Pentelow (1936), using water softened by replacing calcium ions with sodium ions, and Wingfield (1940), using water hardened by adding calcium chloride, detected no differences between the growth of 8-month-old trout in these solutions and in natural hard and soft waters, respectively. Differences in ionic composition of natural waters are typically associated with differences in fauna and hence in food supply.

Salmonids which migrate to the sea show a great increase in growth

rate in the salt water; again, this may be due to differences in food supply rather than to differences in water composition.

The total salt concentration must affect the osmoregulatory position of the fish (see Chap. IV) and any extra stress may well affect growth adversely.

D. Space Factors

Space factors might affect fish in two different ways: (1) the total volume of water containing the fish might influence growth, (2) the degree of crowding of the individuals (volume of water per fish) may be important.

Comfort (1956) gives growth curves for female guppies, *Lebistes*, living alone in containers of different sizes. The fishes attained "specific sizes" characteristic of the conditions used; on similar feeding schedules, they grew larger in the larger containers. When transferred from a smaller to a larger container, the guppies resumed growth and soon reached the specific size characteristic of their new conditions.

Willer and Schnigenberg (1927) found that groups of trout fry grew more rapidly when the total volumes of their troughs and their surface areas were large than when either or both of these were reduced. This effect began to operate only when the fry began to feed.

When several fish are held in the same tank, they are likely to come into contact with each other and may form such associations as size hierarchies (p. 372). If the total space available is rather small, the fish must inevitably disturb each other when feeding and during their normal activity. In still water, or water circulating slowly, there is also the problem of accumulation of waste products and insufficient aeration may lead to death of susceptible species.

Brown (1946b) grew groups of 2-year-old *Salmo trutta* in tanks of different sizes and with different numbers of individuals. There was no apparent effect of absolute size of tank on rate of growth but there was an optimum degree of crowding. Very crowded fish with only 3 liters per fish, and uncrowded fish with 50 liters per fish, grew more slowly than fish with 12, 23, or 35 liters per fish. The overcrowded fish ate less, used the food less efficiently, and clearly disturbed each other more than less crowded individuals; the fish with much space fed and grew erratically and it seemed that a certain amount of "social stimulation" was conducive to rapid growth. Welty (1934) noted that individuals of *Notropis*, *Macropodus*, *Brachydanio*, and *Carassius* ate more food when grouped but grew faster when isolated.

An optimum degree of crowding also affects the growth of trout fry (Brown, 1946a). Maximum productivity was obtained in 8 months from

a 7-liter aquarium by starting with 80 alevins rather than with 25, 50, 100, or 150 alevins.

Allee and his collaborators (see, e.g., Allee, Finkel, and Hoskins, 1940) have explained the existence of an optimum degree of crowding by postulating a "conditioning of the medium" by the fishes. Goldfish, *Carassius auratus,* secrete a substance which has growth-promoting properties; it can be extracted from the surface of the fish, eluted, concentrated, and diluted and it is effective in very low concentrations. In very crowded tanks, the accumulation of waste products and mechanical disturbance overrides the growth-promoting effect so that the fish grow less well. Brown could not detect any "conditioning" effect with tanks of 2-year-old trout.

E. Water Movements

Some species of fishes are characteristic of rapid streams, others of sluggish rivers, and others of still waters, while many may live under varied conditions of water movement. Under experimental conditions, it is usually desirable to keep fishes in still water or water flowing as slowly as is compatible with good survival. There are, as yet, no data to show whether the degree of water movement may affect the growth rate; maintenance requirements may well be higher for fish living in rapid currents rather than in still water.

VIII. GROWTH HORMONES

The pituitary gland of fishes secretes a growth-promoting hormone (see Chap. VI). Pickford (1954) injected purified hormone (mostly from hake, *Urophycis tenuis*) at a dosage level of 10 μg. per gram into hypophysectomized male *Fundulus heteroclitus* which were kept at 20° C. with 8 hours of light per day. The average growth increments were 26.3% for weight and 4.0% for length in 8 weeks, whereas hypophysectomized fish with no injections did not grow in length and showed erratic changes in weight. For similar individuals injected with purified beef growth hormone at the same level, the average increments were greater (39.5% for weight and 7.3% for length), but there were differences in other substances present in the two hormone preparations and the beef preparation showed thyrotropic activity.

Swift (1954) found that 4-year-old *Salmo trutta* responded to purified mammalian growth hormone.

IX. POND CULTURE

Since fish provide a source of animal protein, their "cultivation" is a matter of considerable importance in many parts of the world, particularly where other sources of animal protein are restricted. Fish culture

is an ancient tradition in the Far East and eastern Europe; it has become increasingly important in tropical regions of Africa during the last twenty years and experiments are still in progress to determine the species to be used and the conditions most advantageous for them in

TABLE IV

THE ANNUAL PRODUCTION OF FISH IN VARIOUS WATERS [a]

WATER OR COUNTRY	Main Crop	Yield in pounds/acre (approx. = kg./ha.) Live Weight
UNFERTILIZED WATERS		
Austrian alpine lakes	Salmonids	1.2–6.5 [b] means
Baltic Sea, 1922	—	4 [b]
Swiss and German alpine lakes	Salmonids	12.9 mean
English and Welsh fresh waters	Salmonids and coarse fish	20.6
Lake Tiberias, Israel	*Tilapia*	22.5
German ponds	Carp	22.5
Lake Mendota, United States	Carp and others	22 [b]
North Sea, 1922	—	24 [b]
Iceland Fishing Grounds, 1922	—	34 [b]
Ponds in Alabama, United States	Bluegills	97–125
Brackish water ponds, Indonesia	*Chanos*	40–300
Lake Waubesa, United States	Carp	400
Brackish water ponds, Philippines	*Chanos*	400–980
Brackish water pond, Singapore	*Tilapia*	1200
Tropical lakes	—	2000
Ponds near hot springs, Java	Carp and others	2000–10000
FERTILIZED PONDS		
Germany	Carp	100–400
Alabama, United States	Bluegills	252–432
Yugoslavia	Carp	366 [b]
United States	Bluegills, etc.	300–450
Israel	Carp	1820 [b] mean (4732 [b] max.)
Japan	Carp	3600–5400
Hong Kong	Mainly carp	2000–4000
China	Mainly carp	2800–6000
Malaya	Mainly carp	3500
Java	Mainly carp	4000
Belgian Congo	*Tilapia*	5135–9000 [b]
ANIMAL CROPS ON LAND FOR COMPARISON		
Cattle on cultivated land		84 [b]
Pork on cultivated land		500 [b]
Beef on grassland		5–250

Carp—*Cyprinus carpio*, bluegills—*Lepomis macrochira*.

[a] From Mortimer (1954), Tables I and II.

[b] Kilograms/hectare.

these territories (see, e.g., De Bont, 1954). Much valuable information has also been collected in the United States.

The species to be cultivated, the types of ponds to be used, and the desirability of adding chemical substances and forage must depend on local conditions. The very extensive literature has been collected, discussed, and partially summarized by Mortimer (1954). Table IV has been extracted from this paper and it illustrates the possibilities of production of fish in different parts of the world.

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Chapter X

THE BIOCHEMICAL COMPOSITION OF FISH

R. M. Love

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I. INTRODUCTION

Determination of the biochemical composition of fish appears at first sight to be a simple problem, requiring only patience and accuracy for its solution. It soon becomes apparent, however, on reading the literature, that much work still remains to be done, and that many analyses done in the past have been wasted effort as far as present-day investigators are concerned. Although thousands of data already exist on water, protein, fat, and ash in fish, it is significant that at least one project is now in progress to re-examine a few fish for just these components (Stansby, 1954). The reason is simply this: although earlier investigators often indicated that analytical figures could vary enormously with age, sex, or

season, few seemed able to provide data that took such factors into account. Consequently, average values may have very little meaning, referring as they often do to just one particular batch of fish. Even Atwater (1892), who performed extensive and careful analyses on fifty-three species of fish, regarded his work as only a beginning, owing to an insufficient number of samples. Subsequent reviewers have often ignored this fact, and have indiscriminately quoted values derived from one or two fish as being typical of the species (Stansby, 1954). Caution is therefore needed in interpreting much of the published data.

Vinogradov (1953) points out that of the 20,000 or so known species of fish, only about 350 to 400 of them, mostly marine, have been submitted to chemical analysis, and since the choice of species has usually been limited to those of commercial importance, a balanced picture of the composition of fish as a whole has not so far been achieved. Further, the different parts of the body have not received equal attention. If one compares published tables of composition of fish with those of, say, rats, it is noticeable that in most papers on fish, only the musculature is analyzed, while in rats the composition of all the organs, i.e., brain, spleen, etc., is often given. This seems to be because fish are usually analyzed from the standpoint of food technology or nutrition, and consequently such detailed analyses as those of Suyama and Tokuhiro (1954) on many organs of fish, are rare. For the same reason, there are many tables of composition of canned, salted, and even cooked fish, which are not of primary interest to the fisheries biologist, neither are the data of investigators who did not record what material was used—whether, for instance, the whole fish was analyzed, or just the fillets, or if "fillets" included skin or remnants of bone.

Many analyses have been done on fish "as purchased," i.e. after being caught commercially and stowed in ice for several days. The composition can alter considerably under such conditions, either from chemical or bacterial action, or by the leaching out of constituents by the melting ice. Some data of this kind is given by Chatfield (1954), who reports the vitamin C concentration of several fish as "negligible" although appreciable amounts are, in fact, present in fresh fish tissue (see references in Table I, p. 413).

In the following pages, the factors which affect the concentration, real and apparent, of fish constituents will be presented more fully.

II. RELIABILITY OF METHODS

While the principal need for caution in interpreting published data arises from variations in the fish themselves, examination of methods commonly used reveals a remarkable diversity in detail, which leads to

a corresponding diversity in results. Some inconsistencies shown in the analyses of the most frequently investigated constituents are described below.

A. Determination of Proteins

Fish proteins have almost always been determined by nitrogen analyses using Kjeldahl's procedure, of which little will be said except that faulty digestion, probably common in earlier work, is the greatest source of error in the method itself (Kirk, 1947). Apart from that, the chief error in protein estimations is the factor by which nitrogen values are multiplied. Atwater (1892) tested the conventional factor of 6.25 against "protein" obtained by difference (total weight minus water, fat, and ash) and found it to be nearly correct apart from some exceptional cases, notably skate (*Raia*). Now this fish is known to be rich in non-protein nitrogen (Shewan, 1951) and, since Atwater was estimating nitrogen in whole muscle, this component would be included. Other earlier workers who found that the factor gave rise to incorrect answers, probably for the same reason, were Johnstone (1918) and Bruce (1924). Recently Brandes and Dietrich (1954) proposed a factor of 5.72 for herring, in order to take the nonprotein nitrogen into account. This new factor presumably does not apply to other species, however, and since constituents of the nonprotein nitrogen vary seasonally (Ronold and Jakobsen, 1947; Jones, 1954), it is undoubtedly more satisfactory to isolate the "pure" protein first by precipitation with, for example, trichloroacetic acid.

When this is done, the factor 6.25 is still unsatisfactory, according to the work of Bailey (1937) and Nottingham (1952), who show that 6.025 is much nearer the truth for fish, in which case most published values on fish protein content are rather high.

B. Fats

Results obtained by different investigators are not always comparable, even though the Soxhlet continuous-extraction apparatus is almost universally used. While results from fatty fish are fairly consistent, those from fish of low fat content usually vary with different fat solvents. Such solvents as ethanol–ether mixture, which are effective in splitting the fat from the protein with which much of it is combined, unfortunately dissolve nonfatty substances also, giving very high results. Conversely, solvents such as petroleum, which dissolve only fat, give low results because of their poor "wetting" power and inability to split the fat–protein linkages. The most reliable technique is probably therefore to extract the tissue with a good "wetting" solvent, such as ethanol–

ether mixture, evaporate off the solvent, and extract the residue with petroleum ether, followed by further purification (Lovern, 1955). Seeler and Dietrich (1951) found that if fish tissue was digested with hydrochloric acid before extraction with ether, the figures were more reproducible and greater than those obtained from simple continuous ether extraction. Presumably the acid served the same purpose of splitting the fat from the protein.

C. Water

Atwater (1892) regarded water as one of the most difficult components to determine accurately, and when we consider the widely differing techniques used by various authors, it becomes clear that few results are strictly comparable. For instance, Brandes and Dietrich (1953b) found differences of up to 1.57% between determinations done at 60° C. *in vacuo* and those done at 96° C. A few examples of conditions that have been used will effectively illustrate the difficulty of comparing results: samples have been dried at (1) 60° C. *in vacuo* (Brandes and Dietrich, 1953b); (2) 70° C. (Lühmann, 1952); (3) 96° C. in a current of dry hydrogen (Atwater, 1892); (4) 97–102° C. mixed with baked sand (Youdanova, 1939), and 110° C. (Ahmad, Karim, and De, 1953). The toluene distillation method of Dean and Stark (1920) is also widely used. The significance of the bound water which is only released at the higher temperatures is not clear, and some standardization of the term "tissue water" is needed.

D. Other Substances

In determinations of ash, the chief source of error is probably the use of salted or dried fish, or the inclusion of skin and bones in the determinations, without recording the fact. Such lapses naturally affect the validity of other determinations also.

Biological and microbiological assays are often used for the determination of vitamins, yielding results that are at best approximate. Thoroughly tried methods for the more recently discovered vitamins are rare, so determinations on the same sample by several methods are preferable, as in the paper on nicotinic acid by Ghosh, Nandi, and Banerjee (1951).

When elements occur in fish as part of more than one complex molecule, determinations *in toto* are meaningless to the biochemist. This is so in the case of phosphorus, for example, which occurs in high-energy phosphates, phosphoproteins, nucleic acids, etc., making total phosphorus determinations pointless. Similarly, sulfur is not found as such in fish, but it occurs as methionine and cystine sulfur (Beveridge, 1947), so

that figures for total sulfur have less significance than those showing how much was derived from each component. Similar remarks apply to total nitrogen determinations.

III. FACTORS AFFECTING COMPOSITION

A. Season and Feeding Habits

Differences in diet depending on the availability of food at different times of the year, and changes in the fish caused by the spawning cycle, have a considerable effect on the tissue components, particularly fat. In fish, as in many other animals, fat is stored to supply energy during food scarcity and reproduction, so that we might expect the greatest concentration at the end of the prolific feeding in the summer, and the least at the end of winter. While this is largely true, the relationship between fat and season is not a simple one, especially because sometimes groups of fish within the same species, notably herrings, spawn at different times of the year. Further, although herrings are usually lean after spawning, and at their fattest at the commencement of rapid gonad development, there is no generally applicable relationship between fat and gonad development (Lühmann, 1953). One reason is that the fat reserves of spring-spawning herring are at their lowest immediately after spawning, because of some earlier depletion during the previous winter, while the reserves of the autumn-spawners are not minimal after spawning, because fat is still needed for the winter ahead. Degree of "ripeness," therefore, is no indication of fat content, unless the spawning time of the race is known. The fat of the sardine varies seasonally, and was found by Oya, Usui, and Sukegawa (1937) to have two maxima (summer and winter), suggesting two spawning races in this species also.

Brandes and Dietrich (1953b) found differences of up to 11% between herrings of the same catch, and of the same sex, length, body and gonad weight, effectively demonstrating the difficulty of obtaining a uniform sample.

In herring, the maxima for the fat contents of the flesh and of the viscera do not coincide; the former is followed by the latter, suggesting that visceral fat serves as a reserve food store and continues to be built up after the flesh has been loaded to capacity (Youdanova, 1939). Foster (1955) observed that herrings landed at Fraserburgh (Scotland) ceased to feed after the gonads had reached a certain size, presumably because of the space they required in the body cavity. This cessation probably accounts for much of the seasonal variation in fat. Mužinić (1931) observed that food intake followed a diurnal rhythm, but since the intes-

tines were consistently full, this would probably not cause hour-by-hour variations in flesh fat.

As there is an inverse linear relation between fat- and water-content in fatty fish (Brandes and Dietrich, 1953b; Tsuchiya *et al.*, 1953), water is also subject to seasonal fluctuations. Published data collected by Vinogradov (1953) shows that there is also a seasonal variation in ash and nitrogen, although, according to Tsuchiya *et al.* (1953), nitrogen does not vary seasonally in the skipper (*Cololabis saira*).

Seasonal variations have in addition been demonstrated in vitamin A (Lovern, Edisbury, and Morton, 1933; Pugsley, 1939; Noguchi and Bito, 1953), nicotinic acid (Klocke *et al.*, 1946), some free amino acids (Jones, 1954), and trimethylamine oxide in herring and sprats (Ronold and Jakobsen, 1947), and cod and haddock (Shewan, 1954).

B. Locality

A few components vary according to the locality of the fishing ground. In some cases the reason is straightforward. For instance, European sprats from certain bays and fjords which are rich in food are often fatter than those from the open sea (Lühmann, 1952). It is not possible, however, to explain Shewan's observation (1951) that Arctic fish contain rather more trimethylamine oxide than those caught in the North Sea, although if the true function of this substance were known, a solution might appear. Baltic Sea herring are known to differ in their main spawning times according to locality (Lühmann, 1953), so that at any given time of the year, different local families will doubtless exhibit differences in components that are influenced by the spawning cycle, notably fat.

C. Heterogeneity of Tissue

It is well known that constituents are distributed differently in different organs—the liver of *Squalus suckleyi*, for instance, contains over 98% of the total vitamin A of the fish (Higashi *et al.*, 1953). What often appears to be overlooked is that the musculature itself is not homogeneous, but alters markedly from head to tail. Figure 1 (Brandes and Dietrich, 1953a) shows that the belly flaps (portion 21) of a herring fillet have the highest concentration of fat, followed by the part just anterior to the dorsal fin (portions 6 and 7); the concentration then diminishes steadily towards the tail, and is also less nearer the head. Behr (1952) showed that in herring the water content of the belly, "back," and tail was 55.6, 58.6, and 64.9%, respectively, i.e. was in inverse proportion to the fat, and similar figures are shown by Brandes and Dietrich (1953a).

There appears also to be a gradation of concentrations from the surface inwards. Foster (1955) found the highest concentration of herring fat to be just under the skin, and Hirao, Yamada, and Kikuchi (1954) demonstrated that there was more vitamin A in the deeper layers than in the surface flesh of salmon, *Oncorhynchus keta.*

Tsuchiya and Takahashi (1950) found that the connective tissue in "tail" muscle was more than double that in the "middle" part, but from data cited by Jacquot and Creac'h (1950), the total protein does not vary much from end to end.

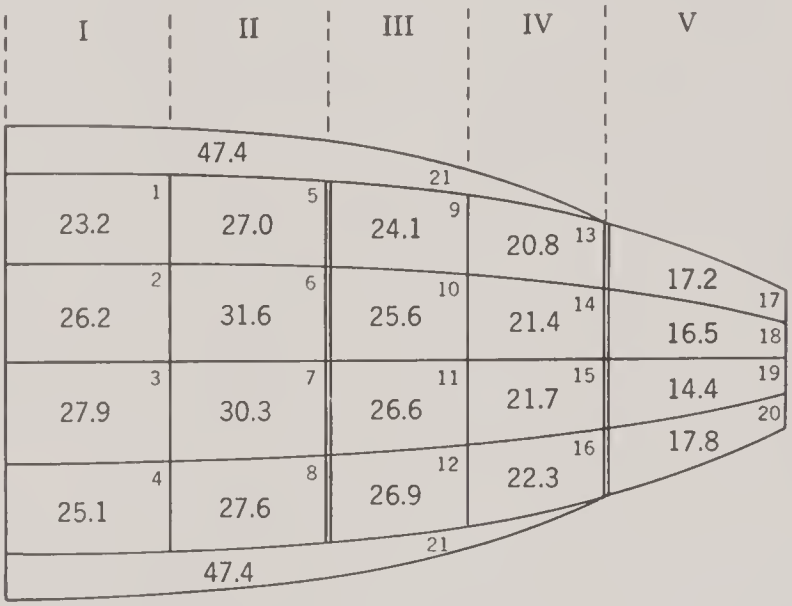

FIG. 1. The concentration of fat in different parts of a herring fillet. Numbers in the top right corners are the serial numbers of the areas analyzed; those in the centers the percentages of fat. Areas 21 are the belly flaps. The posterior margin of II lies just anterior to the dorsal fin. Sections I and V are, respectively, the head and tail end (reproduced from Brandes and Dietrich, 1953a).

The dark flesh along the lateral line of fish differs from the white flesh, containing, for instance, much more vitamin B_{12} (Yanase, 1952), more iron (Parks and Rose, 1933), and less trimethylamine oxide (Shewan, 1951).

All these observations show that the practice of using whole minced fillets for chemical analysis may be misleading, because the composition of such material will vary according to the filleting technique used, on account of the inclusion or exclusion of certain parts of the fish.

D. Size

As fish grow bigger, the percentage of many constituents increases, for example trimethylamine oxide (Shewan, 1951; Anderson and Fellers, 1952), crude ash in the bones (Tsuchiya *et al.*, 1953), fat (Lovern, 1938;

Ono *et al.*, 1953; Tsuchiya *et al.*, 1953), and vitamin A (Lovern *et al.*, 1933; Yamamura, 1952). The linear relationship in the case of deoxypentose nucleic acid and of nonextractable protein is shown in Fig. 2. Total protein is unaffected by the size of the fish (Tsuchiya *et al.*, 1953; Ironside and Love, 1955). *Squalus suckleyi* males contain less vitamin A than the females, but Yamamura (1952) found that this was actually a size effect, the males being the smaller of the two.

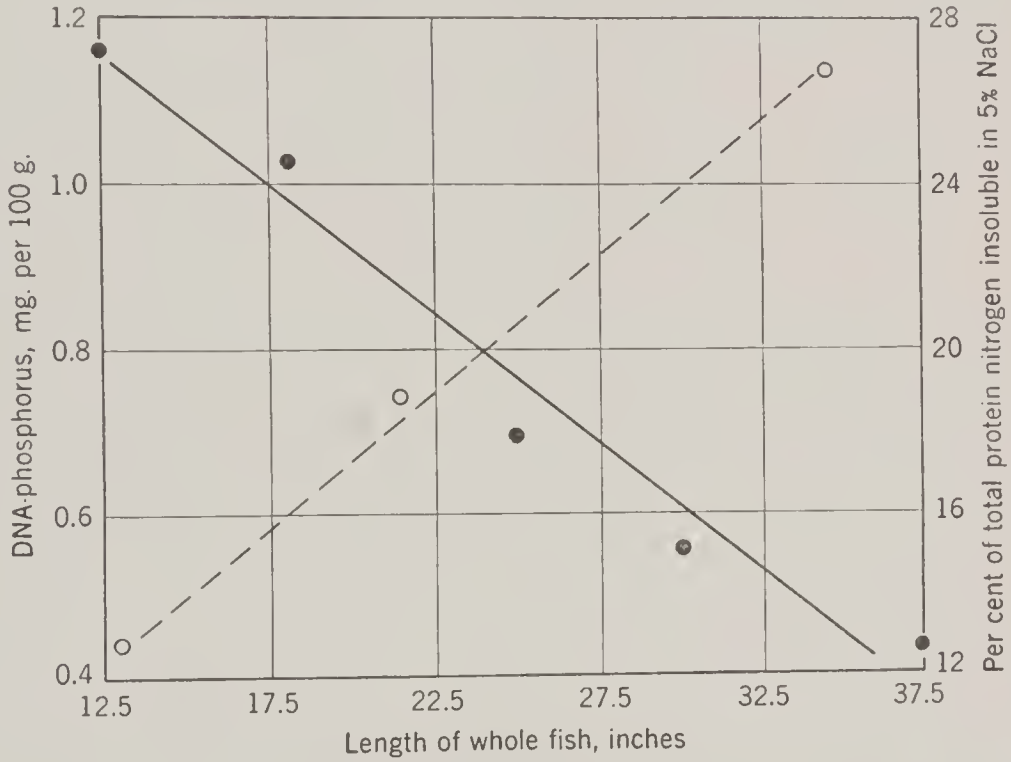

FIG. 2. The relation between fish size and the concentration of two muscle constituents of the cod (*Gadus callarias*) caught in the North Sea. ● — ● — ●: Deoxypentose nucleic acid (DNA) phosphorus in minced muscle taken from "head" ends of fillets of fish caught September 1954 (from Love, 1955). ○ - - - ○ - - - ○: per cent of total protein nitrogen insoluble in 5% NaCl buffered at pH 7.0–7.5, from minced whole fillets of fish caught December 1954 (from Ironside and Love, 1955).

E. Halinity

1. Minerals

Although the minerals in fresh and saltwater fish tissue have often been analyzed, few data exist on the effect of environmental salt concentration on the tissue materials of the same species. Figures collected by Vinogradov (1953) show that when fish migrate from sea to river, there is a fall in total tissue ash. Further, tissue calcium in specimens of *Hypomesus olidus* taken from two Japanese lakes of different salt content was found to be in nearly the same proportion as the calcium in the

lakes themselves (Usui, Sukegawa, and Chou, 1937), showing a clear correlation between composition and environment. It is perhaps premature to generalize, however, since there appear to be no similar data on other minerals so far.

2. Vitamins A_1 and A_2

Broadly speaking, freshwater fish store vitamin A_2, euryhaline fish a mixture of vitamins A_1 and A_2, and marine fish vitamin A_1. There are, however, exceptions which make the above statement an over-simplification. No complete explanation of the occurrence of the two vitamins has been put forward so far. Various possibilities are discussed by Collins, Love, and Morton (1953), but for the purposes of this chapter, it is sufficient to say that the proportions of the vitamins A are constant from species to species, and the mixture found in euryhaline fish remains the same whether the fish is in salt or fresh water.

3. Fats

Marine and freshwater fish each have distinct types of body fat, and the two types are always recognizable, although different species may have their own characteristic fatty acid patterns as well. Species living in estuarine waters or which visit both environments have an intermediate type (Lovern, 1951). Feeding experiments have shown that dietary fats become incorporated into the depot fats of the fish (Lovern, 1938), and this fact, coupled with corresponding differences found in the fats of marine and freshwater plankton (Lovern, 1935), probably explains the two types of fat in marine and freshwater fish.

We should therefore expect the fats of euryhaline fish to alter according to habitat, and this has been shown to be true in the case of young salmon, *Salmo salar,* moving downstream and into the sea. Under such circumstances, the fat changes from a typical freshwater fish fat to the special fat of the adult marine salmon (Lovern, 1934). Thus the composition of fish fats, unlike vitamin A, is influenced by halinity, although the cause in this case is the different foods present in the two habitats rather than the action of the salt itself.

4. Trimethylamine Oxide (see also Chap. IV)

This compound is present in marine fish but much more scanty or absent from freshwater fish. Almost no data exist on the effect of changing salt concentration, but Beatty (1939) found a small quantity of trimethylamine oxide in eels, *Anguilla rostrata,* from the sea, which was not present in those from fresh water. Again, the mechanism is probably one of changed diet rather than direct action of the salt. This

theory is supported by the work of Benoit and Norris (1945), who found no increase in the trimethylamine oxide of young salmon, *Oncorhynchus tschawytscha,* kept in a tank of water, the halinity of which was progressively raised from freshwater to seawater level over a period of 45 days. It could, however, be argued that this period was much too short for one to form any definite conclusions.

IV. NATURE AND AMOUNT OF FISH CONSTITUENTS

A. Proteins

The proteins of fish have been investigated less than those of warm-blooded animals, probably because many research establishments are too far from the sea to obtain fresh material.

1. Connective Tissue

Fish muscle embodies less connective tissue than, for instance, beef muscle, which fact probably accounts for its tenderness. In bony fish, the connective tissue nitrogen is usually 3 to 6% of the total protein nitrogen (Reay, Cutting, and Shewan, 1943), and in elasmobranchs 8 to 10% (Subba Rao, 1948). Tsuchiya and Takahashi (1950) quote figures of 0.16 to 0.80% of actual connective tissue, but these figures are very low, probably on account of the method used. Their connective tissue averaged 78.5% collagen and 21.5% elastin.

2. Soluble Proteins

After submitting extracts of fish muscle to electrophoresis, Connell (1953) found that the resulting diagrams were quite characteristic for each species of fish, so much so that it was possible to identify the latter from them. The diagrams of closely related species showed as much disparity as did the diagrams of unrelated species, but it is interesting to note also that the differences between marine and freshwater fish were no greater than between different species from one habitat. The principal fibrillar proteins, actin and myosin, are very similar to the corresponding proteins in mammalian muscle (Connell, 1954).

B. Fats [1]

The fatty acids present in fish depot fats are straight-chain monocarboxylic acids. Only acids containing an even number of carbon atoms are found, and the chain length may extend from 12 to 26 or 28 carbon atoms. While no one species seems to possess examples of all

[1] The following information is taken from Lovern (1951).

the fatty acids in the range given, fish fats are noteworthy in that they embody a large range of homologues, in contrast, for example to plant fats, where chiefly C_{16} and C_{18} acids are represented, other acids only occurring as traces. About 16% of the fatty acids are saturated, and consist mainly of palmitic acid (C_{16}), with smaller amounts of myristic (C_{14}) and stearic acids (C_{18}). Most of the unsaturated acids are C_{18} and C_{20} derivatives, with smaller amounts of C_{16} and C_{22} derivatives, and only traces of C_{14} acids.

These fatty acids do not occur in the free state, but as glycerides, i.e. as esters on the three hydroxyl groups of glycerol molecules. Unless one fatty acid occurs in overwhelming preponderance, it is most unusual for all three acids on one glycerol to be the same: a mixture is the general rule. Since there are about 20 fatty acids in fish fats, the number of different glyceride combinations is enormous. The mixed triglycerides have similar physical properties, and are difficult to separate; consequently it is impossible to assess the glyceride pattern of such complex fats as those of fish.

C. Water

Fish in general contain about 80 to 85% water, extreme values ranging from 53 to 89.3%. This is higher than in birds (about 70%) and mammals (about 75%) (Vinogradov, 1953). The skin alone also contains more water than the skin of terrestrial animals (Takahashi and Shiokawa, 1953, but the eye lenses contain less (Brückner, 1941; Love, 1954). This will be discussed later.

D. Minerals

It is remarkable that marine and freshwater fish have similar amounts of gross ash in their tissues, usually 1 to 2%. However, many individual elements, such as boron, fluorine, bromine, lithium, strontium, and some others are more concentrated in marine fish than in freshwater fish (Vinogradov, 1953). Parks and Rose (1933) found slightly more manganese in freshwater fish than in marine fish, but such cases are unusual. Fish are also able to accumulate some minerals preferentially, e.g., mercury, which is more concentrated in fish tissue than in fresh or salt water (Raeder and Snekvik, 1941). For a comprehensive treatise on mineral content and distribution in fish tissue, the reader is referred to Vinogradov's work (1953).

V. COMPOSITION AND FUNCTION

Some chemical peculiarities of fish can reasonably be related to their environment or way of life. Thus, it is established that the high urea concentration in the blood of marine elasmobranchs helps to preserve

TABLE I

A Bibliographical Table of the Composition of Fish

Constituent	Number of Species Analyzed	References
Quantitative Analyses		
Amino acids (total)	22	Jacquot and Creae'h (1950)
Amino acids (free)	Cod (*Gadus callarias*)	Jones (1954)
Anserine	10	Yudaev (1950)
Ash (total)	331	Vinogradov (1953)
Betaines	9	Shewan (1951)
Carnosine	10	Yudaev (1950)
Connective tissue	13	Reay, Cutting, and Shewan (1943)
	6	Oya and Kuragake (1935)
Creatine	17	Shewan (1951)
	17	Joshi, Master, and Magar (1953)
Fat (total)	153	Jacquot and Creae'h (1950)
	34	Reay, Cutting and Shewan (1943)
Fatty acids	46	Lovern (1942)
Gases (in swim bladder)	33	Vinogradov (1953)
Histidine	10	Yudaev (1950)
Inositol	11	Shimada and Tanaka (1936)
Mineral constituents	Many species	Vinogradov (1953)
Nicotinic acid	14	van der Rijst (1950)
	4 (freshwater)	Klocke *et al.* (1946)
	24	Joshi, Master, and Magar (1953)
	28 (4)[a]	Higashi and Hirai (1948)
Phospholipid	18[b]	van der Rijst (1950)
	2[c]	Kaueher *et al.* (1944)
Protein or N	153	Jacquot and Creae'h (1950)
	314	Vinogradov (1953)
Trimethylamine oxide	81	Dyer (1952)
	17	Joshi, Master, and Magar (1953)
	9 (freshwater)	Anderson and Fellers (1952)
	2[a]	Suyama and Tokuhiro (1954)
Urea	19 (elasmobranchs)	Smith (1936)
	4[a]	Suyama and Tokuhiro (1954)
	15	Shewan (1951)
Vitamin A	12 (livers); 37 (oil)	Fixsen and Roscoe (1940)
	119 (oil)	Butler (1946)
	18[a]	Edisbury *et al.* (1938)
Vitamin B_1	13	Fixsen and Roscoe (1940)
	26	Sautier (1946a)
	24	Joshi, Master, and Magar (1953)
	21	van der Rijst (1950)
	Salmon[a]	Pyke and Wright (1941)
Vitamin B_2	22	Fixsen and Roscoe (1940)
	26	van der Rijst (1950)
	24	Joshi, Master, and Magar (1953)
	26	Sautier (1946b)

TABLE I (Continued)

A BIBLIOGRAPHICAL TABLE OF THE COMPOSITION OF FISH

Constituent	Number of Species Analyzed	References
Vitamin B_{12}	55	Yanase (1953)
Vitamin B_t (Carnitine)	4	Fraenkel (1954)
Vitamin C	11	Fixsen and Roscoe (1940)
	16	van der Rijst (1950)
	Salmon [a]	Pyke and Wright (1941)
Vitamin D	43	Butler (1946)
Water	153	Jacquot & Creac'h (1950)
	340	Vinogradov (1953)
	3[a]	Suyama and Tokuhiro (1954)
QUALITATIVE ANALYSES		
Constituents of bile	47	Hazlewood and Wootton (1950)
Nitrogenous extractives	9	Shewan (1953)
	10	Shewan (1955)

The mineral composition of the otoliths of *Gadus callarias* and of the scales of 6 species of fish are collected by Vinogradov (1953). Minerals and protein in the scales of one species of shark are given by Takahashi and Yokoyama (1950). Vitamins other than those listed above have been investigated to a far smaller extent, although some data on vitamins B, K, etc., and folic and pantothenic acids, biotin, etc., are given by van der Rijst (1950), Lopez-Matas and Fellers (1948), and Grangaud (1950).

[a] Analyses done on several organs.

[b] These data, taken from (1931 and 1932) papers by Bähr and Wille are listed as "lecithin," but examination of the method shows that they are total phospholipid.

[c] Cephalin, lecithin, and sphingomyelin also given.

osmotic equilibrium and prevent the fish from being dehydrated by the sea water (Baldwin, 1948, and Chap. IV).

The uneven distribution of herring fat shown in Fig. 1 may perhaps be explained in terms of buoyancy. The belly flaps are a suitable site for the storage of fat, since their muscular activity is relatively small, but a high fat content in such a position would tend to make the fish float upside down unless the "back" fat also increased. This might explain the high fat concentration just anterior to the dorsal fin. Again, as the head is not a fat-storage organ, then the tail should not be one either if the fish is to remain horizontal in the water (Foster, 1955).

In view of the higher percentage of water found in fish tissues generally than in mammals, it is significant that the lenses of fish eyes contain much less water than mammalian lenses. When the center parts of the lenses are examined, they are seen to be very hard, and the two facets are probably connected. The hardness appears to be a special feature designed to enable the lenses to retain their shape under rapidly

changing external pressure, as when the fish dives. Mammalian lenses are much more pliable.

Fish show special morphological modifications which fit them for life in the sea. These examples, and there are probably many others, show that they are modified chemically as well.

VI. GENERAL CONCLUSIONS

It is perhaps permissible at this point to make certain recommendations for future studies on the composition of fish, on the basis of the experimental results presented in this chapter.

Assuming that the methods used are the most accurate known, there should then be absolute specificity in naming the fish studied, not giving merely the systematic name, but also the place of catching (to identify local families), approximate time of spawning, and any other characteristic which distinguishes that particular race. It is also essential to give full details of the preparation of the sample. Since several constituents vary in different parts of the fillet, the use of whole minced fillets may be undesirable because of differing filleting techniques, and it is probably better to select a definite part of the fillet—even perhaps a particular segment. Further work is needed to determine the usefulness of such precision. Finally, the normal range of values over one year should be given for at least two age groups. Monthly determinations would probably suffice.

Such conditions are indeed formidable, and emphasize the immense amount of work yet to be done, but future work which does not fulfill them will have little permanent value to other investigators.

VII. THE CONSTITUENTS OF FISH

It can be seen from what has already been said that most published figures give just a rough indication of the true range of values—indeed, several authors admit this to be so—and the fact must be borne in mind when consulting them. The papers listed in Table I represent a large proportion of present-day knowledge of fresh fish composition; some of them are purely reviews, others give original values. The number of species analyzed is given to show the scope of each paper. There are, of course, many more papers giving figures for just one or two species, but the table is intended to show as far as possible the composition of fish as a whole. When more than one author is quoted for one constituent, it is generally because different authors give values for fish from different quarters of the globe, as for example nicotinic acid, which is given for European, American, Indian, and Japanese fish, respectively. Unless otherwise stated, values refer to marine species, or marine with a few freshwater species.

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